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Short Communication

# Production of a fructosyl-transferring enzyme by *Aureobasidium* sp. ATCC 20524

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## SUMMARY

Production of a fructose-transferring enzyme by *Aureobasidium* sp. ATCC 20524 and reaction conditions for the production of fructooligosaccharide, isokestose, were studied. The maximum total enzymatic activity of culture broth was 103.2 U/ml. The optimum reaction pH and temperature of intracellular enzyme was 5–6 and 50°C, respectively.

## INTRODUCTION

Fructooligosaccharides have become important because of their favorable functional properties for foods [3,10]. The industrial production of fructooligosaccharides (isokestose and nystose etc.) has been carried out by fructose-transferring enzymes from *Aspergillus niger* [3–5]. Some microorganisms, for example *Aureobasidium* sp. [4,7], *Fusarium* sp. [1], *Penicillium* sp. [6,11], *Aspergillus* sp. [8] etc. also produce enzymes which produce fructooligosaccharide from sucrose.

In the present paper, we describe the experimental production of fructose-transferring enzyme by *Aureobasidium* sp. ATCC 20524 and the properties of intracellular enzyme for the industrial utilization of the strain to produce isokestose [2].

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## MATERIALS AND METHODS

*Cultivation and preparation of crude enzyme*

*Aureobasidium* sp. ATCC 20524 was used for this investigation. The strain was precultivated (seed culture) in 50 ml of liquid culture medium (sucrose 20%, yeast extract 2%, $NaNO_3$ 1%, $K_2HPO_4$ 0.5%, $MgSO_4 \cdot 7H_2O$ 0.05%, pH 6.5) for 2 days in Erlenmeyer flasks. The cultivation for enzyme production was then started by transferring 2 ml of seed culture to 100 ml medium (of the same composition as the seed culture) and shaking at 30°C for 5 days. The culture broth was centrifuged at 13 000 × *g* for 15 min, and cells were washed two times with water and lyophilized. The supernatant solution and dried cells were used as the extra- and intracellular enzyme, respectively.

*Enzyme activity assay*

The enzyme reaction was initiated by the addition of 0.1 ml enzyme solution (the supernatant solution of culture broth or ca. 20 mg dry cells/ml) to a mixture of 0.4 ml of 75% (w/v) sucrose, 0.5 ml of 0.15 M MacIlvain buffer (pH 5.0). The reaction was carried out at 50°C for 30 min and terminated by boiling for 10 min. To the reaction mixture, 5 ml of 20% sulfosalicylic acid dihydrate was added and filtered through millipore filter (pore size, 0.45 μm).

Sucrose, glucose and fructose content in the reaction mixture were measured using HPLC (Nippon Bunko type 801-SC) fitted with Wakopack WB-T-130E (7.8 mm OD × 300 mm) column under the following conditions: temperature, 60°C; mobile phase, water; flow-rate, 0.2 ml/min; and detector, Erma Optical Works RI detector model ERC-7520. One unit of fructose-transferring enzyme ($U_{FT}$) was defined as the quantity of enzyme responsible for the transfer of 1 μmol of fructose in 1 min. Similarly, one unit of hydrolyzing enzyme ($U_H$) was defined as the quantity of enzyme responsible for the release of 1 μmol of fructose from sucrose in 1 min.

*Preparation and analysis of the product of enzymatic reaction*

The oligosaccharide in the reaction mixture was isolated by paper chromatography (*n*-butanol–pyridine–water = 6:4:3) and lyophilized. This preparation, which showed a single peak in HPLC analysis, was used to determine the chemical structure. Molecular weight of the preparation was measured using a Hitachi Perkin-Elmer 115 molecular weight apparatus. The measurement conditions were as follows: the main room temperature was 60°C, the sub room temperature was 55°C, the solevent used was distilled water. Maltose (Wako) was used for the standard substance.

The preparation was hydrolyzed with 2 N $H_2SO_4$ for 30 min at 110°C, then neutralized with $BaCO_3$ and analyzed by HPLC to determine the ratio of fructose to glucose. The preparation was also digested with invertase (Wako) for 30 min at 30°C and pH 5. Conditions of methylation, GC-MS and $^{13}C$-NMR analysis were as previously reported [2].

## RESULTS

*Time course of the production of fructose-transferring enzyme*

The time course of fructose-transferring enzyme production by *Aureobasidium* sp. ATCC 20524 is shown in Fig. 1. From Fig. 1A it can be seen that cell growth reached 21.3 mg dry cells/ml broth on the third day from the start of cultivation and that the maximum specific enzymatic activity of cells was 2.8 U/mg dry cells on the second day. Fig. 1B shows the intra-, extracellular and total activity of 1 ml culture broth. The intracellular enzyme activity increased first followed by an increase in extracellular enzyme after a 1 day lag as the cell content decreased. The maximum total activity was 103.2 (the third day) U/ml broth.

The changes in the content of sucrose, glucose and fructose in the broth during the cultivation are shown in Fig. 2. A 94% decrease in sucrose decrease was noted by the first day, and then glucose and fructose were released.

*Reaction conditions for fructose-transferring enzyme*

The enzymatic reaction conditions of fructose-transferring enzyme were investigated. The effect of pH on the enzymatic reaction is shown in Fig. 3.

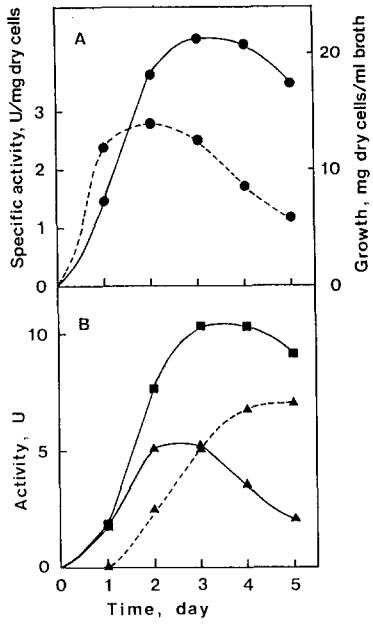


Fig. 1. The time course of fructose-transferring enzyme production by *Aureobasidium* sp. ATCC 20524. Symbols: cell growth, —●—; specific activity, --●--; intracellular activity, —▲—; extracellular activity, - -▲- -; total activity, —■—.

The optimum pH of the intracellular enzyme was 5–6 (Fig. 3A). The efficiency of fructose-transferring activity, $U_{FT}/(U_{FT} + U_H)$, over the range of pH 4.5–7, was more than 90% of maximum (Fig. 3A). The enzyme was stable around the optimum pH 5–6 and retained 90% of fructose-transferring activity (Fig. 3B).

The effect of temperature on the reaction of intracellular enzyme is shown in Fig. 4. The optimum temperature of the intracellular enzyme was 50°C (Fig. 4A). The efficiency of fructose-transferring activity over the range of 30–50°C was more than 95% and was 97% at 40°C (Fig. 4A). The enzyme was stable under 50°C but was inactivated at 70°C (Fig. 4B).

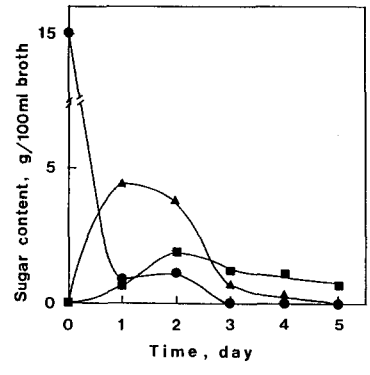


Fig. 2. The changes of sugar contents in the culture broth. Symbols: sucrose, —●—; glucose, —▲—; fructose, —■—.

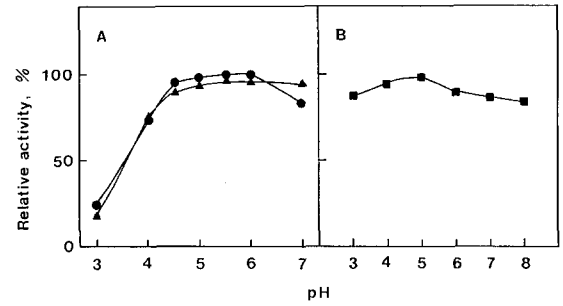


Fig. 3. Effect of pH on the fructose-transferring activity of intracellular enzyme. Symbols: relative activity, —●—; efficiency, —▲—; stability, —■—. The activities were measured after 20 h incuvation at each pH to determine stabilities of the enzyme.

*Analysis and preparation of the product of enzymatic reaction*

Assayed molecular weight (585) and $R_f$ value of paper chromatography (sucrose, 0.57; maltose, 0.47; the preparation, 0.42; maltotriose, 0.36) of the isolated oligosaccharide suggested that the product was a triose. The ratios of fructose to glucose of the acid and invertase hydrolyzed preparation in HPLC analysis were 1.87 and 1.98, respectively. The GC-

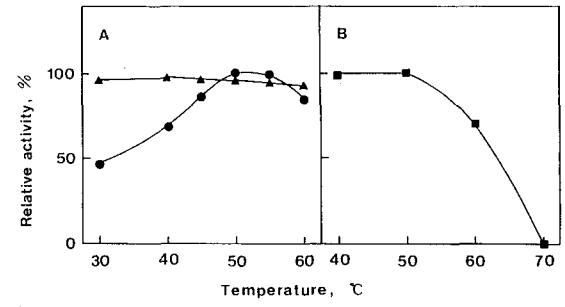


Fig. 4. Effect of temperature on the pH fructose-transferring activity of intracellular enzyme. Symbols: relative activity, —●—; efficiency, —▲—; stability, —■—. The activities were measured after 15 min incuvation at each temperature to determine stabilities of the enzyme.

MS and $^{13}$C-NMR data of methylated preparation were the same to the one previously reported [2]. The results of these experiments suggested that the product was isokestose.

Isokestose produced in the 1 ml of reaction mixture (4.59 $U_{FT}$) at the condition mentioned in Materials and Methods was 48.1 mg when the purified isokestose was used as standared substance in HPLC analysis.

## DISCUSSION

Total fructose-transferring activity of the present strain, 103.2 U/ml culture broth (Fig. 1), was higher than the values of 92.8 U/ml from *Aspergillus* sp. [4] and 90 U/ml of *Aureobasidium* sp. [7]. The specific activity of the present intracellular enzyme, 2.8 U/mg dry cells, also was higher than the values of 0.27 U/mg dry cells of *Aspergillus* sp. and 0.29 U/mg dry cells of *Aureobasidium* sp. [4]. The dried cells of the present strain was very favorable to fructooligosaccharide production because they have very high activity. The pattern of the intra- and extracellular enzymes produced by the present strain was different from that obtained by Jung et al. [7] using *Aureobasidium* sp.

The sucrose concentrations in the culture broth of the present strain (Fig. 2) showed a rapid decrease whereas the decrease by *Fusarium* sp. [1] was gradual. Glucose and fructose concentrations in the broth of *Fusarium* sp. were almost the same and increased slowly whereas glucose concentration in the broth of the present strain was fivefold that of fructose at the first day and then decreased. This suggested that the enzymatic activity of the present strain was too high to supply glucose for the growth. Fructose concentration of the culture broth of *Penicillium* sp. did not decrease but glucose concentration decreased [9]. Thus, the pattern of sugar changes seen with the present strain is very different from those reported for *Fusarium* sp. and *Penicillium* sp.

Optimum reaction pH, 5–6, of the present enzyme (Fig. 3) was the same as that for other strains (e.g. *Penicillium* sp. [9] and *Aspergillus* sp. [4]). Optimum temperature, 50°C, of the present enzymes (Fig. 4) was the same as the enzyme from *Aspergillus* sp. but higher than the enzyme from *Penicillium* sp. [9]. The efficiency of fructose-transferring activity, $U_{FT}/(U_{FT} + U_H)$, of the present strain was considered very high. The efficiency of enzymes from *Aspergillus* sp. was 93.3 and of the enzyme from *Aureobasidium* sp. was 90.6 [4] whereas the efficiencies of the intracellular enzyme (used in this study) at 40°C and 50°C were 97.0 and 95.0 (Fig. 4), respectively. The stability of intracellular enzyme at various pH and temperatures (Figs. 3 and 4) indicate that this enzyme might be used industrially, at factories without adjustment of environment for the reaction. The present enzyme showed the same high specificity of fructose-transferring activity at high sucrose concentration as the other reported enzymes.

We considered that the present strain is potentially valuable as an industrial microorganism because of its high activity of fructose-transferring enzyme to produce isokestose. The scale up, however, of this fermentation for industrial production of fructooligosaccharide remains to be investigated.

## ACKNOWLEDGEMENT

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