World Journal of Microbiology and Biotechnology, 9, 248–250

Development of medium components for the production of glucosyl-transferring enzyme by *Aureobasidium*

S. Hayashi,* T. Hinotani, T. Hayashi, Y. Takasaki and K. Imada

***Aureobasidium* sp. ATCC 20524 produced a glucosyl-transferring enzyme which produced panose (*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose) from maltose. Optimum production for the enzyme was with maltose at 2% (w/v) and yeast extract at 1.5% (w/v). Enzymatic activity reached 0.7 × 10^3 U/g dry cells after 48 h.**

Key words: Aureobasidium, glucosyl-transferring enzyme, maltose, panose, production.

Glucosyl-oligosaccharides, such as panose (*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose), have become important because they support large numbers of *Bifidobacterium* and so are useful in some health-foods (Kitahata 1989). Microbial enzymes have been reported that produce glucosyl-oligosaccharides from maltose by a glucosyl-transferring reaction, for example glucosyltransferase from *Aspergillus* spp. (Pazur & French 1951; Pazur *et al.* 1978; Benson *et al.* 1982; McCleary & Gibson 1989), α-glucosidase from *Saccharomyces* sp. (Chiba & Shimomura 1979), glucoamylase from *Aspergillus* sp. (Vamos & Rose 1973) and *Rhyzopus* spp. (Pazur & Okada 1967) and amylomaltase from *Bacillus* sp. (Pazur & Okada 1968) and *Escherichia* sp. (Kitahata *et al.* 1989). There is, however, no report of the production of glucosyl-transferring enzme by *Aureobasidium*.

In a previous paper, we reported that *Aureobasidium* sp. ATCC 20524 produced β-fructofuranosidases, that had high fructosyl-transferring activity, when it used sucrose as a carbon source (Hayashi *et al.* 1991). Recently, we found that the strain produced a glucosyl-transferring enzyme instead of β-fructofuranosidase when maltose was used as a carbon source. While information on the effect of cultural conditions on the production of glucosyl-transferring enzyme is important for the production of glucosyl-oligosaccharides, little exists. In the present paper, we describe the development of medium components for the optimum production of glucosyl-transferring enzyme by *Aureobasidium* sp. ATCC 20524.

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Materials and Methods

Microorganism and Growth

Aureobasidium sp. ATCC 20524 was maintained on a medium containing (% w/v): maltose, 1; yeast extract, 0.2; agar, 1.8; at pH 7. To optimize the enzyme production, the *Aureobasidium* was grown in 100-ml volumes of medium, which contained 0.5% (w/v) K_2HPO_4, 0.05% (w/v) $MgSO_4.7H_2O$ and various carbon and nitrogen sources (see Results and Discussion), in 500-ml shake-flasks at 30°C for 24 to 120 h. The culture broth was centrifuged and the harvested cells were lyophilized. The enzyme was solubilized from cells with the cell-wall-lytic enzyme Kitalase (2000 U endo-β-1,3-glucanase/g; Wako). Five g dry cells were treated with 20 mg Kitalase in 50 ml of 75 mM citric acid/Na_2HPO_4 buffer, pH 5.0, at 40°C for 2 h and then centrifuged. The resultant supernatant was used as the enzyme preparation.

Enzyme Activity Assay

The enzyme reaction was carried out, in a reaction mixture consisting of 0.2 ml enzyme solution and 0.8 ml 37.5% (w/v) maltose in 75 mM citric acid/Na_2HPO_4 buffer (pH 5.0), at 65°C for 10 min and was terminated by boiling for 10 min.

Products in the reaction mixture were measured by high performance liquid chromatography (HPLC; Nippon Bunko) using a YMC-Pack Polyamine-II column (4.6 × 250 mm; YMC Co Ltd) under the following conditions: temperature, 30°C; mobile phase, acetonitrile/water, 75:25, v/v; flow rate, 1 ml/min; and RI detector. The glucose released in the reaction mixture was assayed

by the glucose oxidase method (glucose test B; Wako). One unit of enzyme activity was defined as the quantity of enzyme responsible for the transfer of 1 μmol glucose in 1 min.

Paper Chromatography

Sugars in the reaction mixture were separated on Toyo No. 50 filter paper using a solvent system of *n*-butanol/pyridine/water (6:4:3, v/v) with three-fold ascents and were detected with silver nitrate reagent.

^{13}C-Nuclear Magnetic Resonance (NMR) Analysis

The ^{13}C-NMR spectra of samples were obtained in solution in D_2O at 250 MHz, with a Bruker A-250 spectrometer operated in the Fourier-transform mode, with complete proton decoupling. Chemical shifts were expressed in ppm from the signal of tetramethylsilane.

Results and Discussion

Enzymatic Properties

The optimum pH value and temperature of the enzymatic activity were 5.0 and 65°C, respectively. The enzyme produced an oligosaccharide from maltose under the reaction conditions described in Materials and Methods. The HPLC retention time of the product (56.5 min) and its paper chromatography R_f value (0.29) were identical to those of panose. The chemical shifts (ppm) from ^{13}C-NMR analysis of the product were also identical to those of authentic panose: 61.2, 61.3, 61.5, 66.5, 70.1, 70.2, 70.6, 72.0, 72.2, 72.3, 72.5, 73.8, 73.9, 74.7, 75.3, 76.9, 77.7, 77.9, 92.6, 96.5, 98.8, 100.4, 100.5. It is suggested that transfer occurs to HO-6, producing panose from maltose.

Effect of Carbon Source on Enzyme Production

Of the various carbon sources used for enzyme production by *Aureobasidium* (Table 1), maltose was the best. The optimum maltose concentration for the production of enzyme by *Aureobasidium* (Figure 1) was 2% (w/v); less enzyme was produced when maltose was at higher concentration.

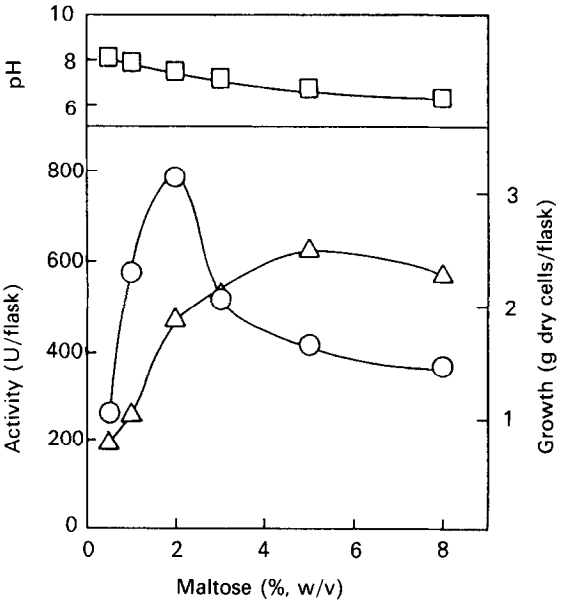

Figure 1. Effect of maltose concentration on the enzyme production by *Aureobasidium*. The basal medium consisted of 2% (w/v) yeast extract, 0.5% (w/v) K_2HPO_4 and 0.05% (w/v) $MgSO_4.7H_2O$. Cultivations were carried out for 48 h and the values given are means from at least two experiments. ○—Activity; △—cell growth; □—pH.

Effect of Nitrogen Source on Enzyme Production

Of the various nitrogen sources used for enzyme production by *Aureobasidium* (Table 2), yeast extract was the best. Cell growth and enzymatic activity were less when ammonium salts, such as NH_4NO_3, NH_4Cl and $(NH_4)_2SO_4$, were used.

The optimum concentration of yeast extract for the enzyme production was 1.5% (w/v) (Figure 2).

Table 1. Effect of various carbon sources on the enzyme production by *Aureobasidium*.*

Carbon source (2.5% w/v)	Final pH	Growth (g dry cells/flask)	Enzymatic activity (U/flask)
Maltose	7.1	2.0	706
Soluble starch	8.0	0.7	219
Galactose	7.1	1.5	116
Sucrose	7.2	1.6	96
Glucose	7.1	1.5	75
Lactose	8.2	0.6	55
Fructose	6.9	1.6	27
Glycerol	8.0	0.7	14

* Basal medium consisted of 2% (w/v) yeast extract, 0.5% (w/v) K_2HPO_4 and 0.05% (w/v) $MgSO_4.7H_2O$. Cultivations were carried out for 48 h. The values given are means from at least two experiments.

Table 2. Effect of various nitrogen sources on the enzyme production by *Aureobasidium*.*

Nitrogen sources (2.0% w/v)	Final pH	Growth (g dry cells/flask)	Enzymatic activity (U/flask)
Yeast extract	7.4	1.9	787
Peptone	6.5	1.3	433
$(NH_4)_2SO_4$	6.5	0.6	39
Malt extract	6.5	0.4	34
$NaNO_3$	6.5	1.3	13
NH_4Cl	6.5	0.6	8
NH_4NO_3	6.5	0.2	6

* Basal medium consisted of 2% (w/v) maltose, 0.5% (w/v) K_2HPO_4 and 0.05% (w/v) $MgSO_4.7H_2O$. Cultivations were carried out for 48 h. The values given are means from at least two experiments.

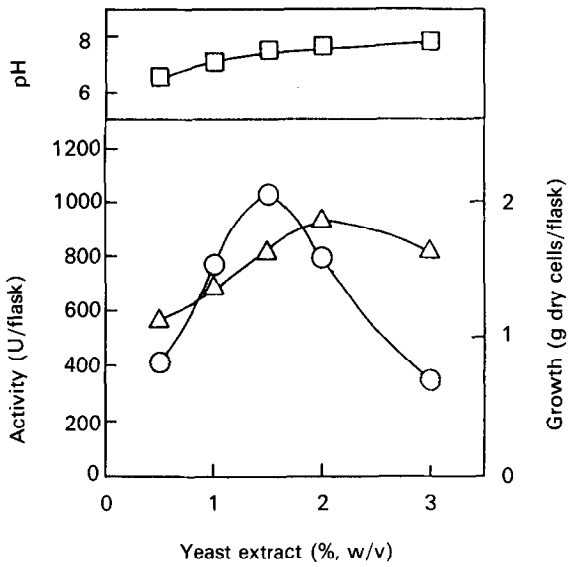

Figure 2. Effect of yeast extract concentration on the production of the enzyme by *Aureobasidium*. The basal medium consisted of 2% (w/v) maltose, 0.5% (w/v) K_2HPO_4 and 0.05% (w/v) $MgSO_4.7H_2O$. Cultivations were carried out for 48 h and the values given are means from at least two experiments. ○—Activity; △—cell growth; □—pH.

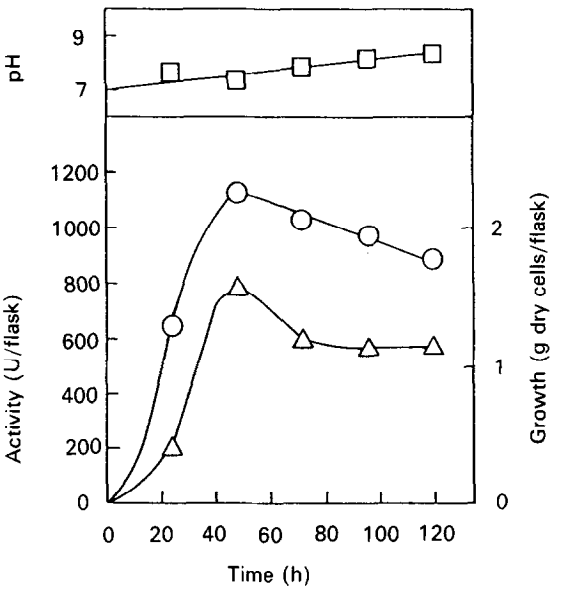

Figure 3. Time-course of the enzyme production by *Aureobasidium* in the optimum medium, which consisted of 2% (w/v) maltose, 1.5% (w/v) yeast extract, 0.05% (w/v) $MgSO_4.7H_2O$ and 0.75% (w/v) K_2HPO_4. The values given are means from at least two experiments. ○—Activity; △—cell growth; □—pH.

The Time-course of Enzyme Production

From Figure 3, it can be seen that total enzymatic activity and cell growth reached 1.1×10^3 U/flask and 1.6 g dry cells/flask after 48 h, respectively. The level of enzymatic activity produced by *Aureobasidium* was very high compared with that of the transglucosidase (1.3 U/ml broth after concentration) previously reported from another micro-organism (McClery & Gibson 1989).

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(Received in revised form 11 October 1992; accepted 26 October 1992)