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NOTE

Effect of Nitrogen Sources on the Production of Polyols by *Aureobasidium pullulans*

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Aureobasidium pullulans produced three kinds of extracellular polyols *e.g.* glycerol, mannitol, and sorbitol from either sucrose, glucose, fructose or mannose. Sorbitol was selectively produced when urea was used as a sole nitrogen source, and the amounts of sorbitol produced rapidly reached a plateau after 50 h where its maximum quantity was about 20 g/l with sucrose.

A series of polyols such as erythritol, mannitol, sorbitol and xylitol have attracted customers in the confectionery and sugar markets due to their desirable characteristics such as being low-calorie, toothfriendly and safe for diabetics (1, 5). They also offers exciting possibilities as alternative sweeteners with bulk properties like various kinds of oligosaccharides (2, 3, 8, 9). Of all polyols introduced so far, apart from erythritol, hardly any microbial polyol products are currently available. *Aureobasidium pullulans* used in this work is already known as a fructo- and isomalto-oligosaccharide-producing microorganism (10-13). In our previous article (14), we described that this strain is capable of transforming some pentoses into glycerol and mannitol. In the course of further investigation, it was found that the carbon and nitrogen source play important roles in the regulation of polyol production in such a way that in a normal growth medium (sucrose and yeast extract medium), glycerol and mannitol were major polyols while in urea medium sorbitol was a sole polyol product.

In the present study, the effect of nitrogen sources on the extracellular polyol production by *A. pullulans* is described.

MATERIALS AND METHODS

Materials

Carbohydrates for substrates and other chemicals used were of reagent grade.

Cultivation of Microorganism

Aureobasidium pullulans KFCC 10245 was cultivated at 28°C in a 250 ml flask containing 50 ml of basal medium composed of 10% (w/v) carbon sources (sucrose, fructose, glucose, and mannose), 0.5% nitrogen sources (yeast extract and other nitrogen sources, see Table 1 for details), 0.2% K_2HPO_4, and 0.15% $MgSO_4$ (pH 6.0).

Analytical Methods

At the predetermined time, 1 ml of the fermentation broth was taken and centrifuged (12000×g). The extracellular polyols were determined by analyzing the supernatants while intracellular polyols were determined after dissolving the packed cells into 3 ml of deionized water, and then treatment using 0.1 g of lysozyme, Kitalase (Kumiai-kagaku, Japan) at 45°C for 1 h. Polyols were identified by HPLC by comparison with the retention times of an external standard, and quantified in relation to the internal standard. In addition, thin layer chromatography was also conducted using a solvent system composed of methyl ethyl ketone-acetic acid-water (9 : 1 : 1 by volume). Polyols and sugars were detected with silver nitrate. Small quantities of intracellular polyols which temporarily accumulated during the early growth phase were neglected.

Sugars and polyol products were analyzed by HPLC (Varian, USA) using the Aminex HPX-42C column (0.78×30 cm, Bio-Rad, USA) and refractive RI-4 detector (Varian, USA). The column temperature was kept constant at 85°C. Water was used as the mobile phase at a flow rate of 0.6 ml/min. Glycerol, mannitol and sorbitol were monitored at the retention time of 19.2, 20.3 and 23.6 min, respectively.

RESULTS AND DISCUSSION

Effect of Nitrogen Sources

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Table 1. Effect of nitrogen sources on the production of polyols by *A. pullulans*[a].

Nitrogen sources	Polyols (g/l)			Residual sugars (g/l)
	Glycerol	Mannitol	Sorbitol	
Yeast extract	11.41	9.22	trace	7.6
$(NH_4)_2SO_4$	9.67	2.45	trace	8.32
NH_4NO_3	4.12	ND[b]	ND	13.81
$NH_4H_2PO_4$	7.55	ND	ND	11.78
Asparagine	4.03	ND	ND	16.98
Urea	ND	ND	17.89	23.41
$NaNO_3$	10.38	5.22	ND	15.11
NH_4Cl	7.11	ND	ND	17.54

[a]Initial concentration of the nitrogen source, 5 g/l; incubation period, 120 h. [b]ND refers to "not detected".

To investigate the effect of different nitrogen sources on polyol production patterns, eight major nitrogen sources were examined using 50 g/l of fructose as a sole carbon source. As shown in Table 1, the trends of polyol production were quite different with the nitrogen sources used. Glycerol was usually produced in all nitrogen sources except urea while sorbitol was selectively produced in the urea medium only. Compared with other nitrogen sources, a low growth rate was achieved in the urea medium, furthermore, only 60% of the initial sugars were utilized. It appears that urea acted as an inducer of the sorbitol-producing enzyme, but simultaneously acted as a growth inhibitor causing slow growth rate of the microorganism. This result is comparable with that a xylitol-producing yeast *Candida* which used urea as the nitrogen source for maximum production of xylitol (4).

Although the carbon skeleton of sorbitol was shown to derive exclusively from fructose, some possible enzymatic routes for sorbitol production have been suggested in *Zymomonas mobilis* (6, 7). Therefore, four possible sugars were tested for sorbitol production. Fig. 1 shows the time course of sorbitol production when sucrose, fructose, glucose and mannose are used as the carbon sources, respectively. For all sugars examined, similar profiles were observed; that is, sorbitol production reached a plateau at the early stage of the fermentation period, thereafter the production of sorbitol remained at a constant level to the end of fermentation. Until the final stage, there also remained a considerable amount of the sugars which were initially employed.

The formation of sorbitol by microorganism is a topic that has not received much attention; it has generally been assumed that reduction of glucose or fructose by a nicotinamide nucleotide-linked polyol dehydrogenase would be responsible. At present it is unclear whether sorbitol production seen in this work is a result of co operative actions such as glucose-fructose oxidoreductase, glucose dehydrogenase, etc. or from other catalytic roles not discovered so far.

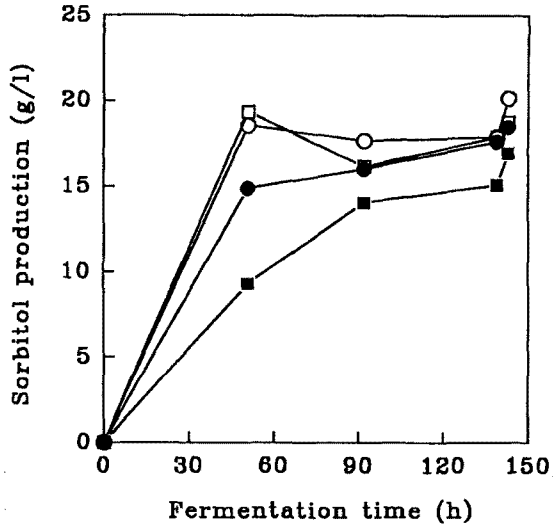

Fig. 1. Effect of carbon source (50 g/l) on the extracellular sorbitol production: (○) fructose, (●) glucose, (□) sucrose, (■) mannose.

In conclusion we would like to stress that *A. pullulans* has many interesting characteristics: it is capable of producing not only two different oligosaccharides as mentioned earlier but also two important polyols *e.g.* mannitol and sorbitol. However, the detailed physiological behaviour of the enzyme system in *A. pullulans* remains in doubt.

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