NOTE

Fermentation Conditions for Panose Production by a Mutant of *Streptomyces hygroscopicus* subsp. *limoneus***Mohammad Mahfuzul Hoque¹ and Eiji Higashide***

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Effect of some inorganic salts, carbon and nitrogen sources on panose production in the former medium¹⁾ by strain GT-32 was investigated. In the medium when 0.3% $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ was added after removing NH_4Cl and NaCl , the strain gave a higher panose production (relative productivity; around 610%) than that of the former medium studied. With the optimum fermentation conditions for panose production, the highest production titer (9.8 mg/ml) was given in 5 days cultured broth.

During the course of study on the screening of new mutants of validamycin (thereafter abbreviated as VM) producer *Streptomyces hygroscopicus* subsp. *limoneus*²⁾, a mutant non-producer (strain GT-32) of VM was found to produce an unusual oligosaccharide. The oligosaccharide was identified as panose¹⁾. Panose was found to inhibit the biosynthesis of the water insoluble glucan from sucrose by *Streptococcus mutans*. Therefore, it might be useful in controlling dental caries formation³⁾. This saccharide is also useful as a growth factor for *Bifidobacterium* sp. in human intestine, suggesting a benefit to the human health⁴⁾. A commercial process of enzymatic production of panose from starch has been established, which needs nine steps. The production of panose by strain GT-32 was 1.59 mg/ml of the broth in a medium originally used¹⁾. We want to achieve production of more amount of panose by strain GT-32. In this report, we describe the development of a suitable medium and optimal fermentation conditions for higher yields of panose than in the medium reported earlier¹⁾.

The maintenance of strain GT-32 and media used for the seed culture and the fermentation

were described in previous papers^{1,5)}. The fermentation medium was supplemented with various inorganic salts. The basal fermentation medium consisted of 2% glucose, 4% corn starch, 2% corn steep liquor (C.S.L.), 4% corn gluten meal (C.G.M.), and 1.5% CaCO_3 . The fermentation medium (30 ml/200 ml flask) was inoculated with 5 ml of two days grown mycelia of GT-32 in seed culture medium consisted of 3% glucose, 2.2% soybean, 0.3% peptone and 0.4% CaCO_3 . pH was adjusted to 7.0 before autoclaving in both fermentation and seed culture media. The inoculated flasks were incubated at 37°C for seven days in a rotary shaker at 180 rpm. Maltose, maltotriose and panose were determined as TMS derivatives by GLC⁶⁾ and also compared with authentic maltose, maltotriose and panose by paper chromatography as described earlier¹⁾.

For investigation on the composition of the production medium, a variety of media containing glycerol, glucose, maltose, or corn starch as a carbon source and soybean flour, yeast extract, beef extract, peptone, C.G.M. (corn gluten meal), or C.S.L. (corn steep liquor) as a nitrogen source were compared for panose produc-

Table 1. Production of panose by strain GT-32 and its growth on various media in 5 days cultured broth

Parameters		Media							
		I	II	III	IV	V	VI	VII	VIII
Components (%)	Glycerol		5.0						
	Glucose	2.0		2.0	5.0	2.0	2.0		
	Maltose			3.0				2.0	
	Corn Starch	4.0				4.0			2.0
	S.B.F.		0.5		1.0				
	Yeast Extract			0.5					
	Beef Extract		0.5	0.5					
	Peptone		1.0	1.0	0.5				
	C.G.M.	4.0				4.0	4.0	4.0	4.0
	C.S.L.	2.0			3.0	2.0	2.0	2.0	2.0
	NH ₄ Cl	0.5					0.5	0.5	0.5
	NaCl	1.5		0.2	0.3		1.5	1.5	1.5
	CaCO ₃	1.5	0.5	0.3	0.5	1.5	1.5	1.5	1.5
pH		8.60	5.69	8.71	6.25	8.19	7.81	8.39	8.30
Growth		4+	+	+	2+	2+	3+	3+	4+
Panose (mg/ml)		1.60	ND	0.10	ND	0.30	ND	0.70	ND

S.B.F., soybean flour; C.G.M., corn gluten meal; C.S.L., corn steep liquor; ND; Not detected

tion and growth of the producer. All samples were collected from 5 days cultured broth. The results are shown in Table 1. A better growth (4+) of strain GT-32 and higher panose production (1.60 mg/ml) were found in medium I. The findings shown Table 1 (medium I) suggested that as for the yield of panose glucose plus corn starch, and C.G.M. plus C.S.L. were notably superior to the other carbon and nitrogen sources, respectively. The compositions of medium I were the same as described in the previous report¹⁾.

Iwasa, *et al.*⁵⁾ carried out an experiment to examine the effect of various inorganic salts including some metal salts on validamycin

production by a VM producer and they found a small but significant effect of inorganic salts except NH₄Cl plus NaCl. This finding led us to carry out similar type of test with medium I as reference. We tested various inorganic salts which are shown in Table 2. We modified the previous fermentation medium with 0.3% MnSO₄·4H₂O instead of NH₄Cl plus NaCl. We found a marked stimulatory effect of MnSO₄ on panose production in the fermentation medium. As in Table 2, MnSO₄·4H₂O (0.3%) added to the medium increased panose production more than 6 fold compared with that in the medium containing NH₄Cl plus NaCl. Manganese is an activator of many enzymes. When Manganese

Table 2. Effects of various salts on panose production by strain GT-32 in 5 days cultured broth

Salts	Conc. in medium (%)	pH	Growth ^b (mg/ml)	Panose (mg/ml)	Relative productivity (%)
NH ₄ Cl + NaCl	0.5 + 1.5	8.60	240	1.60	100.00
NH ₄ Cl	0.5	7.71	210	0.00	0.00
NaCl	1.5	7.96	220	1.58	98.75
FeCl ₂	0.5	7.65	190	1.91	119.78
MnSO ₄ ·4H ₂ O	0.5	8.75	250	9.19	574.38
MnSO ₄ ·4H ₂ O	0.3	10.49	260	9.78	611.25
KCl	0.5	7.46	210	1.67	104.38
ZnCl ₂	0.5	6.42	130	0.00	0.00
No addition ^a	0.0	8.19	160	0.25	15.63

^a Culture broth consisted of 2% glucose, 4% corn starch, 2% corn steep liquor, 4% corn gluten meal, and 1.5% CaCO₃. ^b Wet mycelial weight (W.M.Wt.).

is present in superoxide dismutase, it helps the enzyme to detoxify toxic forms of oxygen, for which cell destruction by super oxide radical was reduced and better growth was attained⁷). Manganese when comes in contact with glucosyltransferase potentates the enzyme activity increasing the production of panose⁸). When we supplemented the enzyme mixture with 1 mM/ml of MnSO₄·4H₂O, it was found that the production of panose was increased (relative activity 121%) than that of the reaction mixture without MnSO₄·4H₂O (relative activity 100%), suggesting that manganese might activate the enzyme reaction *in vivo*. The above individual and/or combined roles of manganese might contribute to the increased production of panose

by strain GT-32 in the medium containing MnSO₄·4H₂O. This finding correlated with the findings of Hayashi, *et al.*⁸).

Optimum pH before autoclaving, fermentation temperature, and volume of the fermentation medium were determined as 7.0, 37°C, and 30 ml / 200 ml flask, respectively. Time course of panose production in the modified medium under optimal fermentation conditions showed the highest production of panose (9.8 mg/ml) in 5 days cultured broth as shown in Fig. 1. Strain GT-32 could also produce maltose and maltotriose in the modified medium. As in Fig. 1, maltotriose production became the highest (10.17 mg/ml) in two days cultured broth and then decreased with an increase of maltose

Fig. 1. Time course of panose production by strain GT-32 in the newly developed medium.

production in the culture broth. On the other hand, maltose production became the highest (13.24 mg/ml) in three days cultured broth and then decreased with an increase of panose production. It appears that there was a possible relation among the three products. We proposed that maltose might be produced from starch and/or maltotriose from which panose could be produced and accumulated in the fermentation medium.

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