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The production of a lactitol-hydrolyzing enzyme from *Escherichia coli*.

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INTRODUCTION

Lactitol (4-*O*-β-D-galactopyranosyl-D-glucitol) is commercial sugar alcohol produced and used as a sweetening agent. It is manufactured from the disaccharide lactose by the reduction of one glucopyranosyl unit to glucitol (sorbitol), the sugar alcohol of glucose. On hydrolysis, lactitol produces equimolar amounts of glucitol and galactose[1].

The reasons for using sugar alcohols as sweetening agents in various foods may be both technological and physiological. Lactitol is a little sweeter than lactose. In contrast to lactose, lactitol is a non-reducing compound and, therefore, inactive in the Maillard reaction, which reduces protein quality and changes the colour of the product. Another physiological benefit of using lactitol as a sweetening agent is its lower cariogenic effect. Sugar alcohols are important as sweetening agents in foods consumed by diabetic subjects. Lactitol, for instance, produces lower levels of plasma glucose than does sucrose[2].

It is well known that *Escherichia coli* induce β-galactosidase (E.C.3.2.1.23), which hydrolyzes lactose under lactose-present conditions. But corresponding information has not been published on the effect of lactitol on the growth of *E. coli*. The purpose of this research is to investigate whether a lactitol-hydrolyzing enzyme is produced, when *E. coli* is cultivated on lactitol as the only carbon source.

MATERIALS AND METHODS

Bacteria strain. The following *Escherichia coli* strains used in this study were from the culture collection of our laboratory: *E. coli* 1008, *E. coli* 1017, *E. coli* 1018, *E. coli* 1076, *E. coli* 1099 (= FAD K-12) and *E. coli* 1166.

Chemicals. Lactitol was kindly provided by Towa Chemical Industry Co., Ltd. (Shizuoka, Japan). Lactose and galactose were purchased commercially from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). Polypeptone (digest of casein) was purchased commercially from Nihon Pharmaceutical Co., Ltd. (Tokyo, Japan). *Saccharomyces fragilis* β-galactosidase (partially purified, Sigma, St. Louis, USA) and *E. coli* β-galactosidase (Grade X, Sigma, St. Louis, USA) were purchased commercially.

Cultivation. *E. coli* strains were routinely cultured in a complex medium (pH 7.0) containing polypepton at 12 g liter^{-1}, lactitol (or lactose) at 10 g liter^{-1} and NaCl at 5 g liter^{-1}. The bacteria were cultured for 24 or 48 hours in shake flasks (500- ml Sakaguchi flasks with a working volume of 250 ml) at 37 ℃. For inoculation, 1% inocula of the bacterial cultures were used. The cultivation of inocula was performed for 24 hours at 37 ℃ in Erlenmeyer flasks (100-ml Erlenmeyer flasks with a working volume of 30 ml).

Preparation of bacterial crude cell-free extracts. *E. coli* cells were collected by centrifugation at 5,800 *g* for 30 min (4 ℃) and washed with 0.01 M sodium phosphate buffer (pH 7.0). The cells were suspended in 10 ml of the same buffer, frozen and stored at −40 ℃ until the next operation. The frozen cells were thawed with running water, and were disrupted by ultrasonication with a sonifier (Branson model 450) for 20 min at 15%

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out put. The supernatant obtained by centrifugation at 27,700 *g* for 30 min (4 ℃) was used as the cell-free extract.

Analytical methods. To assay hydrolase activity, the reaction mixture of 0.5 ml of 0.5% substrate (lactose and lactitol), 0.4 ml of 0.1 M sodium phosphate buffer (pH 7.0), and 0.1 ml of an appropriate concentration of cell-free extract was incubated at 37 ℃ for 10 min. The measurement of galactose was performed using the β-galactose dehydrogenase method (F-kit, D-lactose/D-galactose; Roche, Darmstadt, Germany)[3]. One unit of enzyme activity was defined as the amount of enzyme which produced one μmol of galactose per min. Growth of a bacillus was estimated by measuring turbidity ($O.D._{650}$). When value of turbidity exceeded 1, culture solution was diluted with distilled water and measured.

RESULTS AND DISCUSSION

Growth of lactitol.

When six *E. coli* strains were cultivated on lactitol and lactose, all strains showed good growth (Table 1). In particular, four strains, 1008, 1018, 1076 and 1099, grew better when cultivated on lactitol than on lactose. Therefore, these six strains were thought to produce a lactitol-hydrolyzing enzyme. Accordingly, lactitol-hydrolyzing enzyme production was then examined using all six strains.

Table 1 Comparison of growth of *Escherichia coli* cultivated on lactitol and lactose as the carbon source for 24 hours.

Strain No.	Growth ($O.D._{650}$)	
	lactitol	lactose
E. coli 1008	3.171	1.911
E. coli 1017	1.756	2.268
E. coli 1018	5.166	3.486
E. coli 1076	1.953	1.848
E. coli 1099	2.205	1.638
E. coli 1166	1.365	1.512

Enzyme production.

Figure 1 shows the growth and enzyme activity curves of *E. coli* strains on the complex medium containing lactitol. The enzymatic lactitol- and lactose-hydrolyzing activity was detected in the cell-free extracts. No extracellular enzymes were detected (data not shown). The difference appeared in the enzyme activity level of the strains, *E. coli* 1008 and 1018, which had showed good growth, also showed high lactose- and lactitol-hydrolyzing activity, and others showed only low activity. *E. coli* 1008 grew rapidly even 12 hours, and enzyme activity also increased rapidly. Growth and enzyme activity were peaked at around 12 hours. On the other hand, *E. coli* 1018 continued growing until 36 hours, and enzyme activity also continued increasing parallel with growth. Although the growth of the other four strains was around 50% compared to that of *E. coli* 1008 and 1018 values, enzyme activities were much lower than 50% of the value of strains 1008 and 1018.

The hydrolyzing activity on lactitol was lower than that on lactose in all strains. The maximum activity of each strain on lactitol is summarized in Table 2. *E. coli* 1008 and 1018 showed the highest levels of lactitol-hydrolyzing enzyme of all strains. When the relative value of lactitol-hydrolyzing activity to lactose-hydrolyzing activity was calculated, the percentages of 1008 and 1018 were 20% and 26% respectively. However,

Table 2 Summary of enzyme activities from *Escherichia coli*.

Strain No.	Cultue hours	Enzyme activity (U/ml)		lacti/lacto*** (%)
		lactitol*	lactose**	
E. coli 1008	48	0.54	2.7	20
E. coli 1017	12	0.11	0.37	30
E. coli 1018	36	0.87	3.3	26
E. coli 1076	48	0.16	0.82	20
E. coli 1099	48	0.40	0.60	67
E. coli 1166	36	0.40	0.43	93

*: for lactitol; **for lactose; ***: lactitol-hydrolyzing activity / lactose-hydrolyzing activity.

for *E. coli* 1099 and 1166 which showed low lactitol-hydrolyzing activity, this rate was 67% and 93%, and was higher than those of other strains. *E. coli* 1166 showed a unique result in that "lactose-hydrolyzing activity" and "lactitol-hydrolyzing activity" was almost the same. It is necessary to purify the enzyme from *E. coli* 1166, and to clarify the enzyme's specific activity.

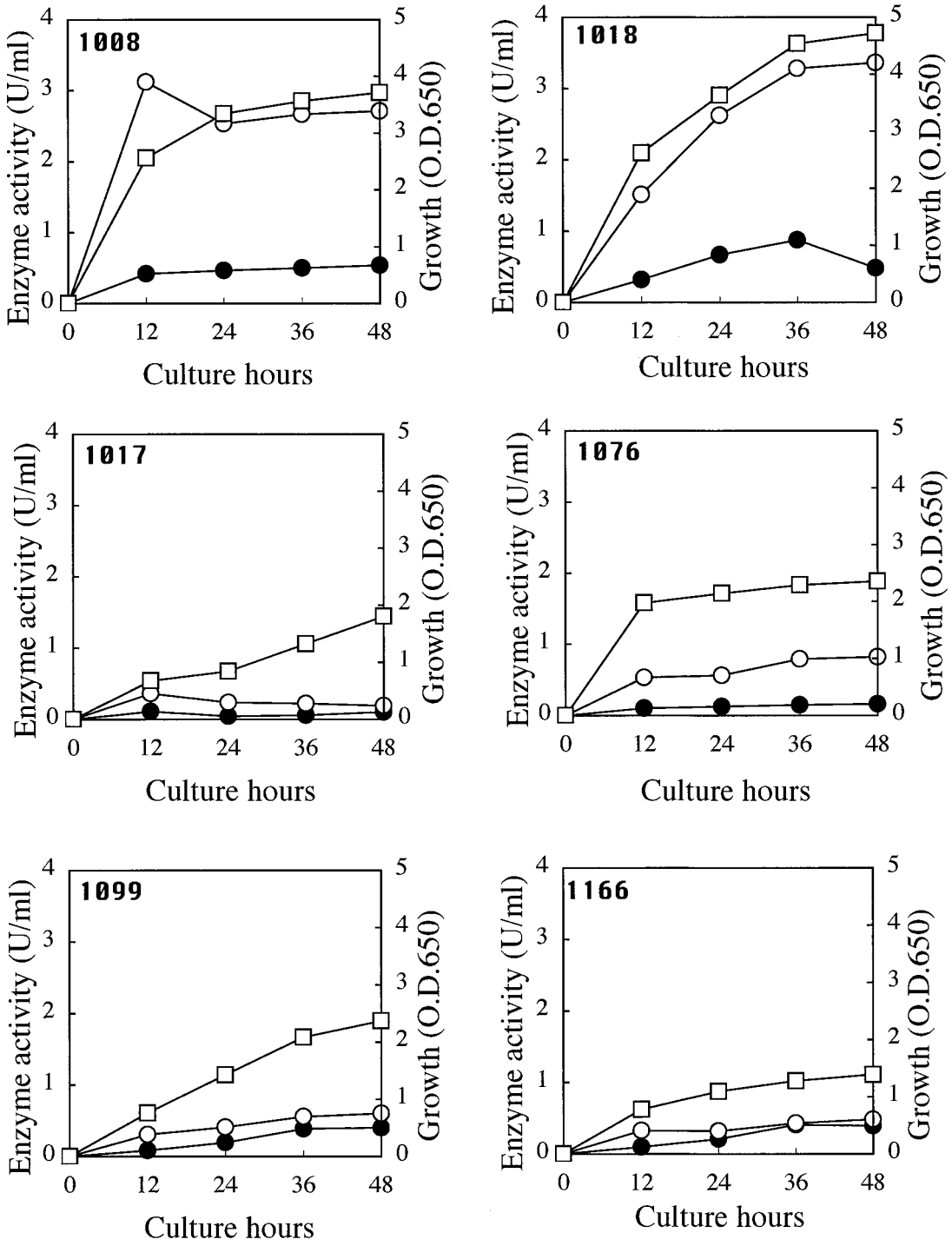

Fig. 1 Time course of growth and enzyme activities of *Escherichia coli*.
Cultivation procedures and measurement of enzyme reaction are described in Materials and Methods.
The numbers 1008, 1017, 1018, 1076, 1099 and 1166 appearing on the graph represent the *Escherichia coli* strains.
□: growth as indicated by turbidity of 650 nm; ●: lactitol hydrolysis; ○: lactose hydrolysis.

Table 3 Comparison of enzyme activities with commercial β-galactosidase preparations.

Preparation	Enzyme activity (U/ml)		lacti/lacto*** (%)
	lactitol*	lactose**	
E. coli 1018	0.87	3.3	26
E. coli 1166	0.40	0.43	93
Saccharomyces fragilis[a)]	0.19	5.0	3.8
E. coli[b)]	2.9	72	4.0

[a]: *S. fragilis* (=*Kluyveromyces marxianus*) β-galactosidase, Sigma, partially purified; [b]: *E. coli* β-galactosidase, Sigma, grade X.
*: for lactitol; **: for lactose; ***: lactitol-hydrolyzing activity / lactose-hydrolyzing activity.

Of currently known enzymes, that with the greatest lactitol-hydrolyzing activity is considered to be β-galactosidase (E. C. 3.2.1.23), and we investigated the lactitol- hydrolyzing activity of commercial β-galactosidase. Commercial β-galactosidase of yeast (*Saccharomyces fragilis*, previous name; *Kluyveromyces marxianus*, present name) and *E. coli* origins was used, and the enzyme activity for lactitol and lactose was investigated. The enzyme activity of the cell-free extract from *E. coli* 1018 and 1166 was compared with that of the commercial β-galactosidase (Table 3). *E. coli* 1018 and 1166 showed low enzyme activity compared with that of commercial β-galactosidase of *E. coli* origin. The relative value of the lactitol- hydrolyzing activity of commercial enzymes was about 4%. However, the relative values of *E. coli* 1018 and 1166 were 26% and 96%, respectively, and were very high compared with those of the commercial enzymes. And these relative values were higher than the already reported values of the cell-free extracts of *Bifidobacterium* and *Lactobacillus*[4)]. This is thought to be due to the fact that our bacteria were cultivated on lactitol instead of lactose. Further research is still needed to cultivate these bacteria on lactose and to investigate the lactitol-hydrolyzing activity of the cell-free extract.

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ABSTRACT

The production of lactitol-hydrolyzing enzymes from *Escherichia coli* was investigated. All six strains used in this study were grown on lactitol as the only carbon source. Hydrolyses of lactitol and lactose were detected in the cell-free extract of all strains. The hydrolysis activity on lactitol was lower than that on lactose in all strains. The highest activity on lactitol was detected in *E. coli* 1018 followed by strain 1008. When the relative value of lactitol-hydrolyzing activity to lactose-hydrolyzing activity was calculated, the highest relative value was 93% for *E. coli* 1166. This value was very high compared with the value of 4% for commercial β-galactosidase of *Escherichia* origin.

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要　　約

大腸菌のラクチトール分解酵素生産を調査した。研究室保存の大腸菌6株についてラクチトール利用性を調べたところ，全ての株で増殖が確認された。

ラクチトールおよび乳糖分解活性は菌体内に認められ，乳糖分解活性に比べラクチトール分解活性は低い値であった。ラクチトール分解活性は *Escherichia coli* 1018 が最も高く，次いで *E. coli* 1098 であった。乳糖分解活性に対するラクチトール分解活性の相対値（%）は *E. coli* 1166 の 93%が最も高く，この値は市販の大腸菌由来の β-ガラクトシダーゼの 4 % と比べ顕著に高い値であった。