食品酵母Candida utilisを用いて調製された短鎖ローカストビーンガムの凍結保護活性

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◇◇◇ Original Paper ◇◇◇

Cryoprotective Activity of Shortened Locust Bean Gum Prepared by Using Food Yeast *Candia utilis*

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Screening various food yeast strains revealed the growth capabililty of *Candida utilis* NBRC1086 on the medium of locust bean gum (LBG). The composition of the LBG medium (pH 5.0) was 0.5% (w/v) LBG, 0.3% (w/v) $(NH_4)_2SO_4$, 0.02% (w/v) $MgSO_4 \cdot 7H_2O$, 0.01% (w/v) Yeast extract, 0.15% (w/v) NaH_2PO_4, 0.1% (w/v) KH_2PO_4. *C. utilis* has been cultivated for 24 hours, degraded by producing the β -mannosidase into the culture broth when transferred to shorten LBG (120 kDa) from LBG (300 kDa). Each LBG could be separated by gel filtration chromatography. The homogeneity of shortened LBG was confirmed by paper chromatography and gel permeation chromatography (GPC). The mannose / galactose ratio in the acid hydrolysate in shortened LBG was found to be 2.8 : 1 using HPLC. The purified shortened LBG had a high level of cryoprotective activity (100%) at a concentration of 50 $\mu g \cdot ml^{-1}$. Furthermore, the activity of the purified shortened LBG was higher than those of LBG, mannan and other sugars. This cryoprotective activity was specified for lactate dehydrogenase (LDH) and alcohol dehydrogenase (ADH). The CP_{50} of purified shortened LBG was 8.3×10 nM, which was almost one-fifth of the CP_{50} of BSA. The cryoprotective activity of shortened LBG against a freeze-labile enzyme, had the possibility of protecting protein component in food materials, *e.g.* eggs, meats *etc.* from freezing damage, then shortened LBG could provide for various frozen foods such as good appetite feelings. The shortened LBG is expected to be more effective as a cryprotectant than LBG for various frozen foods.

Key words: cryoprotective activity; *Candida utilis*; locust beam gum

1. Introduction

The amount of food waste for one year in Japan is 11.4 million ton in 2007, and among them approximately 10% of food waste was the expired food. In order to decrease the amount of food waste by improving the shelf life with the chilled or frozen storage techniques, key materials that have anti-freeze and anti-aging degenerative properties have been desired. One of the mannoproteins from *Pichia anomala* had a high level of cryoprotective activity [1]. Galactomannan that included in the cell membrane of *Saccharomyces cerevisiae*, has shown remarkable effects in the prevention against the frozen degeneration of food materials [1]. Galactomannans are natural nonionic heteropolysaccharide found in the endosperm of certain leguminous seeds [2]. As they can perform storage, water-retaining, and defense function in plants, they are water-soluble and form highly viscous, stable solutions that are widely used as thickeners and stabilizers in the food, cosmetics, and textile industries [3]. Galactomannan consist of a β-1,4-linked D-mannan backbone with a D-galactopyranosyl residues α-linked to mannose C-6 sites. The degree of polymerization of the backbone is 1,000-1,500 mannose units [4]. The individual galactomannans differ from each other in their mannose/galactose residues along the main mannan chain [2].

Among some galactomannans, the most important galactomannans are locust bean gum (LBG) originated from *Ceratonia siliqua* and guargum (GG) originated from *Cyamopsis tetragonolobus*. Mannose/galactose ratios of LBG and GG are approximately 3.5 : 1 and 1.5 : 1, respectively [5]. Bakers and Whistler reported that side-chain units of GG were disposed alternately along the mannan backbone, whereas those of LBG were disposed in uniform blocks [6]. Daas *et al.* suggested that GG has a block wise distribution of galactose side groups and LBG can show random, block wise, and ordered distributions [5].

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These different structures might be caused by the highly viscosity and cryogelation in LBG. This high viscosity was resulted in difficult use at concentrations over 1% (w/v).

Though LBG is usually used as the stabilizers for ice cream, there is no data for the cryoprotective activity of LBG. If the molecular weight and configuration of commercial LBG are controlled, the anti-frozen degeneration properties must have been emphasized. However, dissolubility of galactomannan is too low to control its molecular weight and molecular configuration by using enzyme and chemical reaction. To overcome the defect of this consumption the length of the mannan chain in LBG must be shorteneded using β-mannanase (*endo*-β-1,4-D-mannanase EC. 3.2.1.78) and/or β-mannosidase (*exo*-β-1,4-D-mannanase EC. 3.2.1.25). β-Mannanase, which are isolated from various plants, fungi, and bacteria [7-9], is a hydrolase that catalyzes the *endo*-hydrolysis of β-1,4-mannosidic linkages in the main chain of mannans. It is extremely effective from in safety to use food yeast in a manufacturing process, however β-Mannanase originating from food yeast has briefly been little reported on.

In this paper, we described that the screening of food yeast capable of degrading LBG. We show the purification of shortened LBG and its function as a cryoprotectant having high levels of cryoprotective ability. Furthermore we suggest that shortened LBG had a low level of viscosity by comparison of the viscosity of LBG, and the suggested surface tension.

2. Materials and Methods

2.1 Microorganism and culture medium

The 21 strains of food industrial yeast belonging to the genus *Candida*, 19 strains of the genus *Saccharomyces*, 8 strains of the genus *Zygosaccharomyces*, 4 strains of the genus *Pichia*, 3 strains of the genus *Debaromyces*, 3 strains of the genus *Yarrowia*, 2 strains of the genus *Kluyveromyces*, 2 strains of the *Lipomyceswas*, 2 strains of the genus *Saccharomycopsis*, 1 strain of the genus *Torulospora* and 1 strain of the genus *Yamadazyma* were purchased from NBRC culture collection (Chiba, Japan). One loopful of cells grown on a YM medium plate containing 1.5% agar, medium composition being: 1% D-glucose, 0.5% Peptone, 0.3% Yeast extract (Becton Dickinson, NJ, USA), and 0.3% Malt extract (Becton Dickinson) was inoculated into 10 ml of the YM medium (pH 5.6). The liquid culture was grown on a reciprocal shaker (120 rpm) for 1 day at 25℃, usually in Sakaguchi flasks (500 ml) containing 100 ml of the YM medium (pH 5.6).

2.2 Screening of LBG degrading food yeast and optimum culture condition

The synthetic medium (pH 5.0) containing 0.3% (w/v) $(NH_4)_2SO_4$, 0.02% (w/v) $MgSO_4 \cdot 7H_2O$, 0.01% (w/v)Yeast extract (Becton Dickinson), 0.15% (w/v) NaH_2PO_4, 0.1% (w/v) KH_2PO_4, and 1.0% guar gum, GG (Wako Chemical Co.) or locust beam gum, LBG (Wako Chemical Co.) as carbone sources was used for the screening of LBG degrading food yeast. After 24 h cultivation at a pH of 5.0, each growth and pH were checked. In the medium containing LBG as the sole carbone source, the optimum culture conditions for the production of shortened LBG was confirmed by examining the effect of the concentration of LBG, the initial pH and the culture time.

2.3 Assay of cryoprotective activity against LDH

Cryoprotective activity was measured by a modification of Tamiya *et al.* [10, 11]. A solution of the freeze-labile enzyme, LDH (pig heart LDH obtained from Oriental Yeast Co. Ltd., Japan), was prepared in buffer A (16.66 $U \cdot ml^{-1}$). 20 μl of this LDH solution was kept in a plastic microtube and 80 μl of each sample in buffer A was added. The mixtures were then cooled under controlled conditions ($-1℃ \cdot min^{-1}$) and frozen at -20℃ for 24 h, then thawed by controlled warming ($1℃ \cdot min^{-1}$). The mixture (60 μl) was then added to 3 ml of the assay mixtures (25℃), which contained 80 mM Tris-HCl buffer (pH 7.5), 100 mM KCl, 2 mM pyruvic acid, and 0.3 mM NADH. The decrease in absorbance at 340 nm was measured at 25℃ using a spectrophotometer (U-2001, Hitachi Co., Japan). The residual activity of LDH after the freezing and thawing treatment was estimated by the change in absorbance during the first 2.5 min. One unit of enzyme activity was defined as the amount of enzyme catalyzing the oxidation of 1.0 μmol of NADH per min under the assay conditions. Cryoprotective activity [%] was defined by the ratio of the residual activity to decreased activity after treating with freeze and thawing. Cryoprotective activity [%] was estimated by the following equation.

$$\text{Cryoprotective activity [\%]} = \frac{\{\text{residual LDH activity [U] with sample}\} - \{\text{residual LDH activity [U] without sample}\}}{\{\text{native LDH activity [U]}\} - \{\text{residual LDH activity [U] without sample}\}} \times 100$$

2.4 Analyses of the structure and molecular weight of shortened LBG

The purified shortened LBG was separated using twice Sephacryl S-300 (φ1.6×60 cm) equilibrated with 10 mM

potassium phosphate buffer (pH 7.0) containing 0.15 M NaCl. The purified shortened LBG was hydrolyzed in 2 M HCl solution at 100℃ for 4 h. After hydrolyzing, this hydrolysate was neutralized with saturated $BaCO_3$ and then analyzed using the TOSOH sugar analysis system with a TSK gel Sugar AXG (4.6 mm ID×15 cm). Detection was accomplished by the measurement of the excitation wavelength at 288 nm and the emission wavelength at 470 nm. All sugars in the hydrolysate were converted to fluorescent using the benzamidine and NaOH. The molecular weight of the shortened LBG was estimated with the gel filtration column, Superose 12 (ϕ1.6×100 cm) and Sephacryl S-300 (ϕ1.6×60 cm) using some dextranes having different molecular weight (40–190 kDa). The detections of all sugar amounts were assayed with phenol-sulfuric acid [12]. The high voltage paper chromatography to check the homogeneity of purified shortened LBG was performed by the method described by Fuller [13]. Also, the identification of shortened LBG and the estimations of the molecular weights were performed using Shodex GPC-101.

2.5 Assay of β-1,4-mannase and β-1,4-mannosidase

The β-1,4-mannanase activity in the culture broth was measured by using mannan (*S. cerevisiae*, Wako Co.) as a substrate. One unit of enzyme activity was defined as the amount of enzyme releasing 1 μmol of reducing sugar per min [14]. The β-mannosidase activity in the culture broth was measured by using *p*-nitrophenyl-β-D-mannopyranoside (*p*Np-Man, Sigma) as a substrate. One unit of enzyme activity was defined as the amount of enzyme releasing 1 μmol of *p*-nitrophenol per min [15]. The protein concentration was measured by the method of Bradford's method using bovine serum albumin (BSA) as the standard [16].

2.6 Assay of ice-nucleating activity

The ice nucleating activity was measured using the droplet freezing assay described by Vali [17] with a freezing nucleus spectrometer (thermoelectric plate Mitsuwa model K-1, Yamamoto Technical, Japan). Thirty drops at 10 μl each were placed on a controlled-temperature surface and the temperature was slowly lowered from 10℃ to -20℃ at a rate of 1℃/min. The temperatures required to freeze 10% (T_{10}), 50% (T_{50}), and 90% (T_{90}) of the drops was measured.

2.7 Measurement of surface tension and viscosity

The surface tension of the purified shortened LBG was determined by the pendant drop method at 25℃, which was performed using an apparatus consisting of an automatic interfacial tensionmeter (CBVP-43, Kyowa Interface Science, Japan). A concentration of 1% (w/v) LBG or shortened LBG was dissolved in water. Viscosity in these solutions was performed by using the Brookfield Viscometer OV-1 at 30℃.

2.8 Chemicals

All enzymes, which were highly purified, containing lactate dehydrogenase, alcohol dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, and recombinant isocitrate dehydrogenase were purchased from the company Oriental Yeast Co. Ltd., Japan. The other chemicals were purchased from Wako chemicals Co. Ltd., Japan.

3. Results and Discussion

3.1 Screening of LBG degrading food yeast and the optimum condition for its growth

Using the screening medium containing 1.0% (w/v) GG or 1.0% (w/v) LBG as a sole carbon source, the galactomannan degrading food yeast strain was screened among the 21 strains. Although GG has a high viscosity, *Candida utilis* NBRC 1086 could grow on the GG medium after cultivation for 24 h. Next the effects of LBG concentration, pH, and cultivation time on the growth were examined. The growth of strain 1086 was made quicker by increasing the additional concentration of LBG until raised by only 1% (w/v) LBG. Optical density at 660 nm of the cell that attained its stationary phase after culturing for 20 h was 8.0. However, strain 1086 could not grow on LBG medium containing excess 1.5% (w/v) LBG causing its viscosity and solubility. Among each medium at each pH between 2 and 9, the optimum pH for its growth was 5.0. Judging from the decrease of viscosity in the culture broth and increase of reducing sugar, the optimum culture time for the growth and the production of shortened LBG was 24 hours. Some microorganisms capable of growing in a medium containing galactomannan like LBG could produce only β-mannanase [8,18], or β-mannanase and β-manosidase [9]. After remaining in a centrifuge at 10,800×*g*, for 30 min to remove the cell, the resulting supernatant was examined in the presence of β-mannanase and/or β-mannosidase. Strain 1086 could produce only β-mannosidase in the culture broth, and its

activity in the culture broth was 0.52 U/mg. As β-mannosidase is an *exo*-type enzyme, the remaining LBG in the culture broth might be both whole LBG and shortened LBG.

3.2 Cryoprotective activity of LBG from the culture broth and separation of shortened LBG from the culture broth

One of the glucanase extractable mannoproteins from *Pichia anomala* had a high level of cryoprotective activity [1]. We predicted that mannan-chain had more hydrophobicity than the protein section in its mannoprotein. The resulting supernatant was fractionated using acetone. After dialyzing in distilled water using dialysis membrane (10 kDa-molecular-weight cutoff), the cryoprotective activity of the crude extract was examined. As shown in Table 1, the cryprotective activities of the crude extract and LBG solution, which was dialyzed in distilled water, at a concentration of 100 $\mu g \cdot ml^{-1}$ were 100% and 45.3%, respectively. It was suggested that LBG could hold water in the ice cream as a stabilizer [19]. Next the shortened LBG was separated using Sephacryl S-300 (Fig. 1). The enhanced activity of the shortened LBG was in a fraction indicated by the hooked symbol. This active fraction was pooled and dialyzed in distilled water, and then rechromatographed with the same condition for the isolation of purified shortened LBG. As shown in Fig. 2, the crude extract was separated into two spots that were LBG and shortened LBG. The sample pulled after separation by twice gel chromatography was only one spot. As shown in Figs. 1 and 3, its molecular weight was estimated to be approximately 120 kDa using the Sephacryl S-300 and GPC analysis. Also, the purity of separated shortened LBG could be confirmed by GPC analysis. The production of shortened LBG having this molecular weight might to be caused by the activity of β-mannosidase.

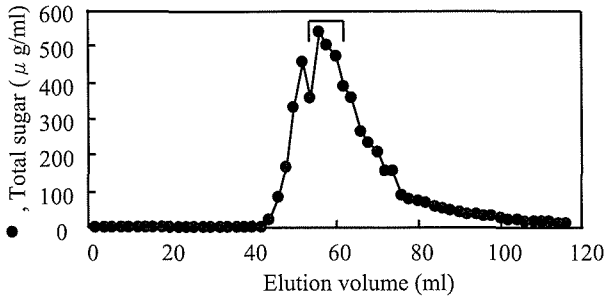

Fig. 1 Separation of shortened LBG using gel filtration chromatography. The hooked symbol indicates the fractions of shortened LBG. The separation was performed with Sephacryl S-300 (φ1.6×60 cm) with 10 mM potassium phosphate buffer (pH 7.0) containing 0.15 M NaCl.

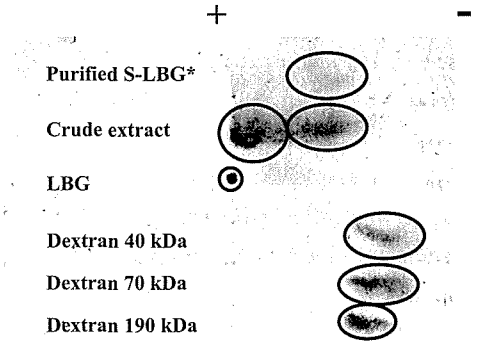

Fig. 2 High voltage paper chromatography of purified shortened LBG.
*Purified shortened LBG was prepared with second gel filtration chromatography.

Table 1 Cryoprotective activities of various sugars.

Compound	Cryoprotective activity (%)
Sucrose	18.2
Galactose*	0
Mannose*	0
Dextran*	17.9
Mannan*	22.0
LBG*	45.3
Crude extract*	100.0
Purified S-LBG**	100.0

Sucrose concentration is 10 mg/ml.
* Sugar concentration is 100 μg/ml.
**Sugar concentration is 50 μg/ml. S-LBG indicates purified shorten LBG.
Mannan is the cell wall component from *S. cerevisiae*.

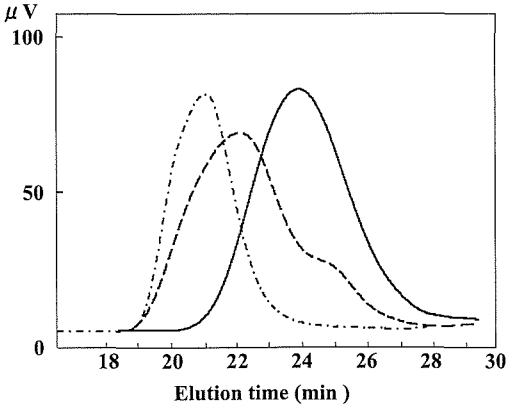

Fig. 3 GPC analysis of purified shortened LBG. Dash-dotted line is LBG, broken line is crude shortened LBG, and solid line is purified shortened LBG.

3.3 Cryoprotective activity and characterization of purified shortened LBG

Fifty $\mu g \cdot ml^{-1}$ of the purified shortened LBG had 100% (Table 1). The effect of concentration on the cyroprotective activity of shortened LBG was shown in Fig. 4. This activity sharply increased over a concentration of $5 \mu g \cdot ml^{-1}$. This activity was not invariable activity over $50 \mu g \cdot ml^{-1}$. The activity decreased 55% at a concentration of $100 \mu g \cdot ml^{-1}$, and then increased again $200 \mu g \cdot ml^{-1}$. This activity curve was the same sigmoid curve as some cyroprotective proteins [10, 20-22]. The interaction of the shortened LBG molecule with each other might differ at $50 \mu g \cdot ml^{-1}$and $100 \mu g \cdot ml^{-1}$ concentrations. Following this, we examined the constituent rate of galactose and mannose in shortened LBG. Through the analysis of acid hydrolyzate using the HPLC system, we found that the ratio of mannose of the shortened LBG to the galactose of the shortened LBG was 2.8 : 1, which was different from that of the normal LBG [5]. This constituent ratio of mannose and galactose has come from the action of β-mannosidase. The cryoprotective activity of purified shortened LBG was higher than those of galactose, mannose, dextran and mannan from the cell wall of *S. cerevisiae* (Table 1). Also, the shortened LBG had no activity (0%) against malate deydrogenase and isocitrate dehydrogenase which are well known as freeze labile enzymes, and this activity was specified for LDH and alcohol dehydrogenase. Furthermore, the CP_{50} of purified shortened LBG was 8.3×10^{1} nM, which was almost one-fifth the CP_{50} of BSA (Table 3). This activity was slightly lower than that (CP_{50} was 2.1×10^{1} nM) of the mannoprotein from *Pichia anomala* [1]. In general, mannnan-chain has the coherent feature such as glucomannan, thereby being weak hydrophobicity. So we examined the surface tensions of LBG and purified shortened LBG. The surface tension of LBG was almost constant at 69 mN/m. In the shortened LBG the surface tension markedly decreased over 2 mg/ml and then this critical micelle concentration was 5 mg/ml. This result suggested that the surface tension of shortened LBG like surface active agent has an important role in expressing cryoprotective activity.

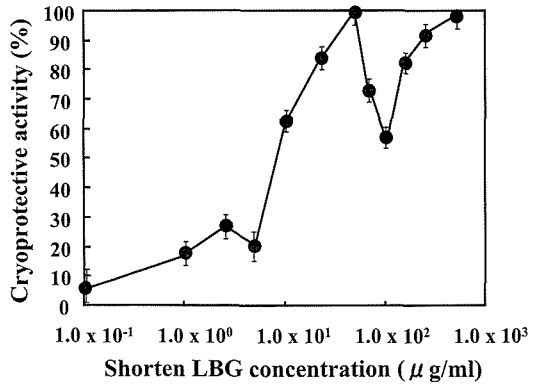

Fig. 4 Cryoprotective activity of purified shortened LBG. Cryoprotective activity was performed by the method described in the section on Methods and Materials. Statistical analysis was performed with a *t*-test. A p value less than 0.05 is considered significant.

GG and LBG, which were consisted of a β-1,4-linked D-mannose backbone with a D-galactopyranosyl residues α-linked to mannose C-6 sites are widely used in the food, cosmetics, and textile industries. GG has a block wise distribution of galactose side groups and LBG can show random, blockwise, and ordered distributions [5]. This structural difference affects the actions of various enzymes, for example, β-mannanase and β-mannosidase. Even though the hydrolyzates of GG using β-mannanase were produced and characterized based on their rheological properties [23, 24], few studies have centered on the hydrolysis of LBG using various enzymes and microorganisms.

As its resulting all LBG was a shortened LBG having a molecular weight of about 120 kDa (Fig. 3), shortened LBG might be produced by the action of β-mannosidase, which was *exo*-type enzyme, in the presence of LBG. In the report of mannosidase from the genus *Candida*, *Candia albicans* could produce soluble α-1,2-mannosidase, which is involved in *N*-glycan processing in this strain [25].

In general, mannnan-chain has the coherent feature such as glucomannan, and has weak hydrophobicity. The shortened LBG would have a higher hydrophobicity than natural LBG, thereby expressing high cryoprotective activity. In order to elucidate the factors in this reduction at a concentration of $100 \mu g \cdot ml^{-1}$ (Fig. 4) the ice nucleation activities of the shortened LBG at each concentration were examined. The ice-nucleating temperature, T_{50} [℃] of the shortened LBG at a concentration of 20, 100, and $250 \mu g \cdot ml^{-1}$ was -13.9, -17.4 and -14.2℃, respectively. Franks reported that the deciding factors in the formation of ice nuclei by materials included the following three conditions: similarity to the ice crystal lattice, the paucity of the surface charge, and the high hydrophobicity of the ice nuclei [26]. The interaction between shortened LBG and ice crystal might be related to this reduction. Furthermore, the purified shortened LBG had the 52 mN·surface tension at a concentration of 5 $mg \cdot ml^{-1}$ (Fig. 5). A mannan-chain of the purified shortened LBG had a better interaction than that of natural LBG, thereby

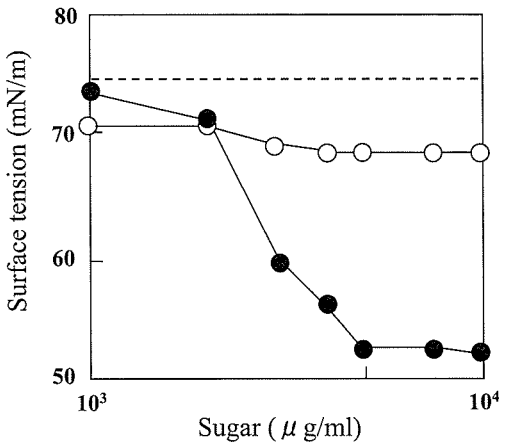

Fig. 5 Effects of the concentration on surface tension.
The broken line shows distilled water. The closed circle shows the purified shortened LBG and the open circle is the natural LBG.

expressing high cryoprotective activity. Tween 80 known to be a detergent had protected the LDH during freezing and thawing [27]. Tween 80 protects LDH from denaturation when freezing and thawing by hindering its destructive interaction with the ice crystal [28]. Protection might be obtained when Tween 80 molecules compete with the protein for sites on the ice surface. The CP_{50} of purified shortened LBG was 8.3×10 nM, which was almost one-fifth of the CP_{50} of BSA (Table 2). The molar ratio of LDH and BSA was 1 : 1.7 for the CP_{50}. This value is similar to that of Tween 80 (1 : 1) [28]. Also, the cryoprotective activity of shortened LBG at 100 $\mu g \cdot ml^{-1}$ under rapid cooling condition in liquid N_2 were 38%. This reduction of the activity was the same as that of Tween 80 [28]. If the protection mechanism of shortened LBG is the same as that of Tween 80, when shortened LBG is used on frozen processed foods, the frozen food might have a subsequently high level of moisture and quality. The viscosity of shortened LBG at a concentration of 1 $mg \cdot ml^{-1}$ was lower than that of natural LBG. By the reduction of viscosity, the solubility of shortened LBG was higher than that of natural LBG (Table 3).

Table 2 Comparions of some cryoprotective matters.

	Molecular weight (kDa)	CP50(μg/ml)	CP_{50}(nM)	Reference
Sucrose	0.34	2.7×10^{5}	8.0×10^{8}	[20]
BSA	66	2.8×10	4.0×10^{2}	[20]
COR15	15	8.3×10^{-2}	5.6×10^{0}	[20]
COR85	350	1.5×10	4.3×10	[21]
COR26	159	1.6×10^{4}	1.0×10^{5}	[29]
HIC6	14.7	1.1×10^{-1}	7.4×10^{0}	[30]
CRP29	29	8.0×10^{-4}	2.7×10^{-2}	[22]
CRP62	62	2.0×10^{-1}	3.2×10^{0}	[31]
AS26k	26	2.0×10	7.7×10^{2}	[32]
COGP	83	1.7×10	2.1×10	[1]
S-LBG*	120	1.0×10	8.3×10	This study

CP_{50} indicated the concentration of each cryoprotectant required to produce a cryoprotective activity of approximately 50%.
* S-LBG indicates purified shorten LBG.

Table 3 Comparison of LBG and shorten LBG.

	LBG	Shorten LBG
Molecular weight (kDa)	300	120
CP_{50} (nM)	6.7×10^{2}	8.3×10
Viscosity (cP)	100.6	33.2

We could find that the shortened LBG produced by *Candida utilis* NBRC 1086 had a high level of cryoprotective activity, and surface tension. Since this shortened LBG has the possibility of holding water, the use of shortened LBG might provide a novel texture for the cryogel in the mixture of other polysaccharide and shortened LBG in future.

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◇◇◇ 和文要約 ◇◇◇

食品酵母 *Candida utilis* を用いて調製された短鎖ローカストビーンガムの凍結保護活性

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種々の食品酵母株のスクリーニングテストによって，*Candida utilis* NBRC1086がローカストビーンガム（LBG）培地で生育できることが明らかになった．*C. utilis* は，0.5％(w/v) LBG，0.3％(w/v) $(NH_4)_2SO_4$，0.02％(w/v) $MgSO_4 \cdot 7H_2O$，0.01％(w/v) Yeast extract，0.15％(w/v) NaH_2PO_4，0.1％(w/v) KH_2PO_4 を含むLBG培地（pH 5.0）で，24時間培養された．また，*C. utilis* は，培地中にβ-マンノシダーゼを生産することによってLBG（300 kDa）を短鎖LBG（120 kDa）に分解していた．各々のLBGは，ゲルろ過クロマトグラフィによって分離された．短鎖LBGの単一性はペーパークロマトグラフィとGPC分析によって確認された．短鎖LBGの酸加水分解物のマンノースとガラクトース比は，2.8:1であった．精製した短鎖LBGは，50 μg/ml濃度で高い凍結保護活性（100％）を示し，LBGやマンナンおよび他の糖よりも高い活性であった．この凍結保護活性は乳酸脱水素酵素とアルコール脱水素酵素に特異的であった．精製短鎖LBGのCP50は，8.3×10 nMであり，この値は牛血清アルブミンの約5分の1であった．また，精製した短鎖LBGは，冷凍液卵を安定化させることも確認できた．我々は，短鎖LBGが多様な冷凍食品に対して凍結保護剤の可能性をもっていると期待している．

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