Comparison of Physicochemical Properties of a Novel Polysaccharide Produced by *Bacillus circulans* with Commercial Polysaccharides

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Physicochemical properties of a new polysaccharide produced by *Bacillus circulans* were investigated by comparing with those of xanthan gum, locust bean gum, guar gum, sodium alginate and carboxymethylcellulose (CMC). The polysaccharide produced by *B. circulans* (BPS) showed a high water-holding capacity at 0.1% concentration together with a good emulsifying effect of oil next to xanthan gum. The viscosity of 1% BPS solution was about 900 cP, the value being nearly equal to that of xanthan gum. The viscosity of BPS solution was almost unchanged at a pH range between 5 and 9, and the heat treatment of BPS at 100°C for 30 min brought about a 25% decrease in viscosity. The addition of 30% glucose to BPS solution enhanced the viscosity by 20%, but the addition of 1% citric acid lowered the viscosity to about 30%. A synergistic effect of BPS on the increase in the viscosity of locust bean gum or guar gum solution was observed. A maximum viscosity of a mixed solution of BPS and locust bean gum or guar gum was obtained at a ratio of about 2 : 8 (v/v). A Young's modulus of a film prepared from BPS was apparently higher than that of CMC and guar gum.

Polysaccharides are present in plants and algae as natural constituents, and also as a product of microorganisms during growth¹⁾²⁾. They are useful as raw materials in food and non-food industry because of their physical characteristics. Some of the polysaccharides originated from plants and microorganisms are used as a thickener and stabilizer of food, edible films, and an additive for a water-holding reagent and stabilizer of non-food materials, etc³⁾. Unlike synthetic polymers, these biopolymers do not cause environmental pollution as they are easily decomposed by microorganisms in natural environment. Especially, polysaccharides produced by microorganisms are paid much attention due to their characteristics and availability to applied fields as compared with polysaccharides derived from plants^{4)~6)}.

In our previous papers, we reported that a

soil bacterium *Bacillus circulans* produced a highly viscous polysaccharide consisting of L-rhamnose, D-galactose, D-mannose, and mannuronic acid or glucuronic acid as sugar components in a medium containing glucose as a carbon source⁷⁾⁸⁾. We concluded that it was a novel bacterial polysaccharide from its chemical composition, and the chemical structure of the polysaccharide is now under investigation.

In this report, we investigated some physicochemical properties of the polysaccharide produced by *B. circulans* (BPS) to examine the possibility of its application to food and non-food industry as raw materials. We chose five kinds of polysaccharide from various sources which have different physical properties according to their chemical structures, and compared their properties with those of BPS.

Materials and Methods

Materials

Xanthan gum from bacteria (*Xanthomonas campestris*), locust bean gum (*Ceratania siliqua*) and guar gum (*Cyamopsis tetragonolobus*) from plants, sodium alginate from brown algae and carboxymethylcellulose were used in this study. Guar gum, locust bean gum and xanthan gum (K and Na form) were kindly supplied by Dainippon Pharmaceutical Co., Ltd., Osaka. Sodium alginate, carboxymethylcellulose and other chemicals were purchased from Nacalai Tesque Inc., Osaka. Locust bean gum was dissolved in distilled water by stirring at 80°C for 15 min, and other polysaccharides were dissolved at room temperature before use.

Culture conditions of bacteria for polysaccharide production

A polysaccharide-producing bacteria *Bacillus circulans* was used in this study⁷⁾. A culture medium of the bacteria for polysaccharide production contained 1% glucose, 0.05% asparagine, 0.005% yeast extract, 0.05% KH_2PO_4 , 0.005% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.005% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.005% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.005% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. The pH of the medium was adjusted to 7.0 before autoclaving at 120°C for 15 min. A jar-fermentor (MSJ-30, Marubishi B.E. Co., Ltd.) culture was done at 30°C for 48 hr with 10 l of the medium by inoculating 1 l of a pre-culture, which was cultured as described in the previous paper⁸⁾, with an aeration rate at 1.5 l per min and an agitation speed at 300 rpm.

Isolation and purification of polysaccharide

A culture fluid obtained by jar-fermentor was diluted with an equal volume of water and centrifuged at 10°C for 20 min at $18\,000\times g$ to remove bacterial cells. To the supernatant solution, 1% cetyltrimethylammoniumbromide solution was added until no more precipitate of polysaccharide-detergent complex occurred. The precipitate was collected by filtration, washed with water, and dissolved in 10% sodium chloride solution by stirring. Two volumes of ethanol were added to the solution, and the resulting precipitate was collected by

centrifugation at $3\,000\times g$ for 10 min, dissolved in distilled water and dialyzed overnight against distilled water at 4°C. The dialyzed solution was lyophilized, and the lyophilized preparation was used for examinations.

Measurement of water-holding capacity

A water-holding capacity of each polysaccharide preparation was measured as follows. A tip of a filter paper (Toyo Roshi No. 51, 2×40 cm) was dipped in 0.1, 0.5 and 1% polysaccharide solutions and kept for 1 hr at room temperature. A moving distance of water from a starting line was measured and a water-holding capacity of polysaccharide solution was expressed as a relative value of moving distance of water to that of distilled water⁹⁾.

Measurement of stability of emulsion

Each polysaccharide solution (5 ml) and salad oil (5 ml, Nisshin Oil Co., Ltd.) were mixed and homogenized with an Ultra-disperser (LK-21, Yamato) for 2 min in a 20 ml-graduated test tube. The mixture was kept for 7 days at room temperature and the stability of emulsion was evaluated by measuring the volume of three layers of emulsion, water and oil^{10) 11)}.

Measurement of viscosity

The viscosity of polysaccharide solution was measured by a B 8 M rotation viscometer (Tokimek) with a HM-1 rotor at 12 rpm (shear rate : 15.8 sec^{-1}) at 25°C unless otherwise stated.

Measurement of Young's modulus of films prepared from polysaccharide

A film of polysaccharide was prepared as follows ; 50 ml of 1% polysaccharide solution was poured into an acrylic plate ($15\times 15\times 0.5$ cm) set at level, and dried in a desiccator with silica gel at room temperature. Prepared films were left at room temperature ($20\pm 0.5^\circ\text{C}$, $60\pm 2\%$ RH) for more than 24 hr. The extension test of films (0.5×5 cm) was performed with a tensile tester (KES-G1, Kato Tech. Co., Ltd.). Young's modulus was calculated from the slope of the stress-strain curve at a constant rate of extension strain (0.002%/sec). The thickness of films was measured at five points on each film (0.5×5 cm) by a compression tester (KES-FB3, Kato Tech. Co., Ltd.) and a mean value was used for calculation.

Results and Discussions

Water-holding capacity of polysaccharide

A water-holding capacity of BPS was compared with some polysaccharide preparations available at the market. As shown in Fig. 1, the BPS solution showed the lowest moving rate of water among the polysaccharides tested at the range from 0.1 to 1.0% concentration, the result indicated that BPS had the highest water-holding capacity among the polysaccharides tested.

Stability of emulsion

Highly viscous polysaccharides are often used as an emulsifier in food-manufacturing industry, for example, xanthan gum is known to be a good stabilizer of emulsion¹²⁾. Then the effect of BPS on the stabilization of emulsion with oil and water was compared with that of other polysaccharides. As shown in Fig. 2, 1% BPS had a maximum effect on the stabilization of emulsion. Xanthan gum was effective at a concentration of 0.5% to give a

Fig. 1 Water-holding capacity of various polysaccharides

Concentration of polysaccharide : □, 0.1% ; ▨, 0.5% ; ■, 1.0%
BPS : Polysaccharide produced by *Bacillus circulans*

Fig. 2 Comparison of stability of emulsion

□, water ; ▨, emulsion ; ■, oil
A, BPS ; B, Xanthan gum ; C, Guar gum ; D, Locust bean gum ; E, Sodium alginate ; F, CMC

complete stabilization. From these results, BPS is effective next to xanthan gum, but more than 1% BPS seems to be required as an emulsion stabilizer.

The effect of addition of sodium chloride or acetic acid to BPS solution on the stability of emulsion was examined. The addition of sodium chloride (2.5%) lowered the stability of emulsion slightly, but acetic acid had no effect on the stability at a final concentration of 10% (data not shown). As the pH of BPS solution containing 10% acetic acid was 2.5, BPS seemed to have a stabilizing effect of emulsion at acidic pH ranges.

Rheological properties of polysaccharides

1) Polysaccharide concentration and viscosity

The relationship between the concentration of polysaccharides and viscosity of their solution is shown in Fig. 3. The viscosity of BPS solution was increased logarithmically with increasing concentration from 0.1% to 1.0% ; 150 cP for 0.5% solution and about 900 cP for 1.0% solution, respectively. A linear change of vis-

Fig. 3 Viscosity change with concentration of polysaccharide

○, BPS; ●, Xanthan gum; ◐, Locust bean gum; □, Guar gum; ■, Sodium alginate; ▨, CMC

Rotor: HM-1 at 3 rpm, viscosity: 10^3 cP >, shear rate: 4 sec^{-1}

HM-2 at 12 rpm, viscosity: 10^3 cP <, shear rate: 4 sec^{-1}

cosity was also observed in locust bean gum, guar gum, sodium alginate and CMC, while xanthan gum was relatively highly viscous at lower concentration than other polysaccharides and the viscosity increased slowly over 0.5% concentration. The viscosity of BPS solution was as high as that of these commercial polysaccharides. A detailed flow behavior of BPS is now under investigation.

2) Effect of pH on viscosity

The effect of pH on the viscosity of polysaccharide solution was investigated. The pH of each polysaccharide solution (viscosity of each solution was set at 125 cP) was adjusted to 2 to 11 with 0.1 N HCl or 0.1 N NaOH. As shown in Fig. 4, the viscosity of BPS solution was nearly independent at the pH range between 5.0 and 9.0, but it decreased rapidly by shifting the pH

Fig. 4 Effect of pH on viscosity

○, BPS (0.40%); ●, Xanthan gum (0.22%); □, Guar gum (0.45%)

The viscosity of polysaccharide solution before adjusting of pH was expressed as 100%.

to acidic or alkaline side. A similar tendency of viscosity change was observed for xanthan gum, CMC and sodium alginate (data not shown). As these polysaccharides have carboxyl residues, the change of viscosity with pH may be due to the alteration of molecular conformation led by the change of a net charge of polysaccharide molecule. This assumption was confirmed by the result that the viscosity was recovered when the pH of polysaccharide solutions with low viscosity was readjusted to 5–9. On the other hand, the viscosity of locust bean gum and guar gum, which are mainly composed of galactomannan, was not affected by the change of pH. This may be due to their neutral molecular structure.

3) Effect of heating temperature on viscosity

The change of viscosity was examined by heating each polysaccharide solution at 100°C for 5–30 min. As shown in Fig. 5, the viscosity of BPS was apparently decreased with heating time. BPS was unstable to heating next to

Fig. 5 Effect of heating time on viscosity of various polysaccharides

○, BPS (0.40%); ●, Xanthan gum (0.22%); ◐, Locust bean gum (0.48%); □, Guar gum (0.45%); ■, Sodium alginate (0.57%); ◑, CMC (1.0%)

The viscosity of polysaccharide solution without heating was expressed as 100%.

sodium alginate. Little change in the viscosity of these heat-treated BPS solutions was observed after they were kept at 8°C for 24 hr, indicating that an irreversible change of molecular conformation of BPS had occurred by the heating. However, BPS solution seems to be stable by heating at 80°C for 30 min (data not shown). The viscosity of guar gum and locust bean gum was increased with heating at 100°C for 5–10 min. This result indicates that the effect of heating may depend on the structural difference of polysaccharide. The increase in the viscosity of neutral polysaccharide (guar gum and locust bean gum) may be due to the progress of hydration by cooling the heated solution.

4) Effect of addition of sugar on viscosity

The effects of addition of glucose on the viscosity of polysaccharide solutions were in-

vestigated. As shown in Fig. 6, the viscosity of BPS, sodium alginate and CMC was increased by adding increasing amount of glucose. A similar effect was also observed when sucrose or fructose was added to the polysaccharide solution (data not shown).

As the viscosity of these sugar solutions was negligible even at the concentration of 30%, the increase in the viscosity by the addition of these sugars may be attributable to the change of conformation of polysaccharide by interaction with added sugar molecules.

5) Effect of addition of organic acid on viscosity

The effect of addition of citric acid on the viscosity of BPS, xanthan gum, locust bean gum and CMC are shown in Fig. 7. The viscosity of BPS solution in the presence of 1% citric acid was decreased to about 30% of that without acid, while that of xanthan gum was about 60% by adding the same amount of citric acid. CMC was less affected in the presence of citric acid. It seems that the viscosity change

Fig. 6 Effect of glucose on viscosity

○, BPS (0.40%); ●, Xanthan gum (0.22%); ◐, Locust bean gum (0.48%); □, Guar gum (0.45%); ■, Sodium alginate (0.57%); ◑, CMC (1.0%)

The viscosity of polysaccharide solution without glucose was expressed as 100%.

Fig. 7 Effect of citric acid on viscosity

○, BPS (0.40%); ●, Xanthan gum (0.22%); ⊗, Locust bean gum (0.48%); □, Guar gum (0.45%); ⊠, CMC (1.0%)

The viscosity of polysaccharide solution without citric acid was expressed as 100%.

of these three acidic polysaccharides may be related to the decrease of pH by addition of citric acid. On the other hand, the viscosity of guar gum and locust bean gum did not change by the addition of citric acid. A similar tendency was also found for these polysaccharides by adding succinic and lactic acid (data not shown). The viscosity of sodium alginate (0.57%) was increased by the addition of 1-2% citric acid, and a gel formation was observed by addition of more than 5% of the acid (data not shown).

6) Synergistic effect of BPS on viscosity of other polysaccharides

It is well known that a solution of xanthan gum forms a gel when it is mixed with locust bean gum, and that the viscosity of xanthan gum solution increases when it is mixed with guar gum¹³⁾. As BPS is an acidic polysaccharide similar to xanthan gum from bacteria, the

effect of addition of BPS on the viscosity of locust bean gum or guar gum was investigated. A solution of locust bean gum or guar gum (each 0.5%) was mixed with 0.5% BPS solution at different ratio, and the viscosity of the mixture was measured. As shown in Fig. 8, the viscosity of a mixed solution of BPS with locust bean gum or BPS with guar gum reached a maximum at the ratio of about 2 : 8 (v/v). It seems from these results that BPS has a synergistic effect to increase the viscosity of locust bean gum or guar gum solution, though the effect was slightly smaller than xanthan gum (data not shown). It is assumed that synergistic effect of xanthan gum on locust bean gum or guar gum results from their similarity of structure with the backbone of β -1, 4 linkage of sugar components. Since BPS showed less synergistic effect than xanthan gum, BPS may have a different backbone structure from locust bean gum and guar gum. The synergistic effect of BPS on locust bean gum or guar gum may be due to the entanglement of molecular chain to form dense network structures. In other experiment, we examined synergistic effect of BPS on viscosity

Fig. 8 Synergism of BPS and other polysaccharides on viscosity

Concentration of polysaccharide : 0.5%

○, LB (locust bean gum); ●, GG (guar gum)

Rotor : HM-2 at 30 rpm, shear rate : 10.2 sec⁻¹

Fig. 9 Young's modulus of films made from various polysaccharides

A, BPS (n=6); B, Xanthan gum (n=3); C, Guar gum (n=10); D, Locust bean gum (n=6); E, Sodium alginate (n=9); F, CMC (n=12). Data was shown by mean values $\pm$ S.E.

Coefficient of variation of film thickness: BPS (39%), Xanthan gum (25%), Guar gum (18%), Locust bean gum (23%), Sodium alginate (28%), CMC (37%)

of xanthan gum. The viscosity of mixed solutions (BPS and xanthan gum) apparently increased as compared with the case of individual solutions (data not shown).

7) Young's modulus of polysaccharide films

Some of the bacterial polysaccharides are used for the preparation of edible films. Therefore, we compared the ability of film formation of BPS with other polysaccharides. All the polysaccharides examined had the ability of film formation. The average of thickness of films were as follows: BPS (80 μ m), xanthan gum (100 μ m), guar gum (350 μ m), locust bean gum (160 μ m), sodium alginate (50 μ m), CMC (70 μ m). Stress and strain of films (0.5 $\times$ 5 cm) were measured and Young's modulus was determined. As shown in Fig. 9, the Young's modulus of these films was in the order of 10^8 N/m². The Young's modulus of

BPS film was 5×10^8 N/m², which was higher than that of CMC and guar gum ($P < 0.05$). The results indicate that BPS is available to film formation as other polysaccharides.

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Bacillus circulans が生産する新規多糖と

市販多糖類の物理化学的性質の比較

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Bacillus circulans の生産する高粘性多糖 (BPS) の物理化学的性質を、キサンタンガム、ローカストビーンガム、グアガム、アルギン酸ナトリウムおよびカルボキシメチルセルロース (CMC) のそれらと比較検討した。

BPS は比較したどの多糖類よりも保水性に優れ、乳化安定性もキサンタンガムについて優れていた。

1.0% BPS 水溶液の粘度は約 900 cP で、他の高粘性多糖と同程度もしくはそれ以上の粘性を示した。

BPS 水溶液の粘性は pH 5~9 でほとんど変化が見られず、80℃ までの加熱には比較的安定であった。また、グルコースの添加により粘度は増大し、クエン酸の添加により粘度が減少した。ローカストビーンガムやグアガムなどのガラクトマンナン系の多糖と BPS との併用により増粘効果がみられた。

BPS はフィルム形成能を持ち、作成したフィルムのヤング率は CMC やグアガムのフィルムよりも危険率 5% 以下で有意に高かった。