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Rheological Properties of Biopolymer Produced by *Pseudomonas delafieldii*

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*Pseudomonas delafieldii*가 생성하는 다당류의 레올로지 특성

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The extracellular polysaccharide was isolated from the culture of *Ps. delafieldii* and its rheological property was evaluated. The aqueous solution was extremely viscous and shows pseudoplastic behaviour. The flow behaviour index and apparent viscosity of 1% solution were 0.09 and 1769 mPa·s. The solution was stable over pH change but did not have thermal stability. The activation energy of flow of 1% solution was 4.44 kcal/mole. The concentration dependency could be expressed double logarithmically.

Hydrophillic colloids, more commonly referred to as gums, are extensively used in industries due to their unique properties rendering the rheological characteristics of aqueous system (1-3). These traditional polysaccharides have been facing an increasing demand and suffering from lacks of reproducibility in quality (4). Since the dextran was manufactured successfully (5), many efforts were made for searching a new microbial polysaccharide (6-8) because the quality and production can be engineered (9,10). In this laboratory, a bacterium with potentiality was isolated and identified as *Ps. delafieldii* (11). The physicochemical properties of its polysaccharide was also previously reported (11). In this paper, rheological properties of the polysaccharide will be discussed.

Materials and Methods

Preparation of polysaccharide

Ps. delafieldii was grown in glucose medium (12) at 30°C after 72 hours in jar fermentor (working volume: 1500 m*l*, agitation: 200 rpm, aeration: 1 vvm, New Brunswick Sci. Co. U.S.A.). After diluting the culture broth and centrifuging at 95,400×g, 2 volume of isopropanol was added to the supernatant and fibrous polysaccharide was harvested. The recovered polysaccharide was redissolved in deionized water and harvested again and freezedried (Model GT-2 Freeze drier, Leybold-Heraeus, Germany).

Analysis

The rheological properties of polysaccharide solutions were examined at 25°C with Brabender Viscotron (Model 80241, Measuring system E-17, Range 10, Maximum shear rate 350 sec^{-1} West Germany) with recorder.

Rheological data were analyzed by fitting to Power law equation (13). The xanthan and guar gum (Sigma chem. co.) were used for comparing the apparent viscosity.

Results and Discussion

Comparison of the apparent viscosity of polysacc-

Key words: *Pseudomonas* sp., polysaccharide, rheology
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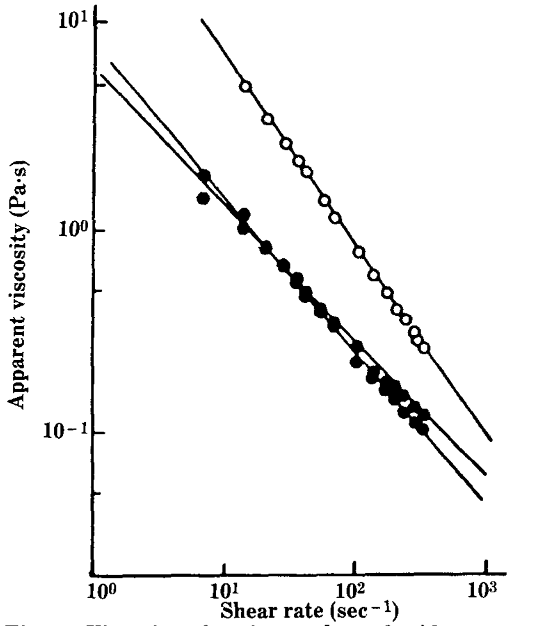

Fig. 1. Viscosity of various polysaccharides versus shear rate.
(○: *Pseudomonas delafieldii,* ● xanthan, ●: guar, concentration: 1%)

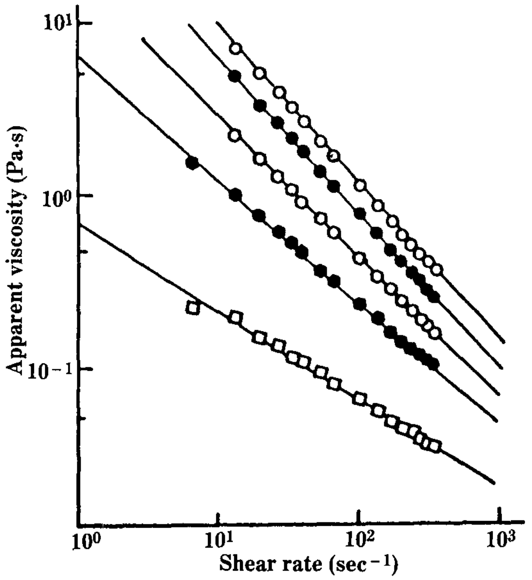

Fig. 2. Concentration dependency of viscosity of polysaccharide produced by *Pseudomonas delafieldii* BT-4.
(○: 1.25%, ●: 1%, ○: 0.75%, ●: 0.5%, □: 0.25%)

haride solution

The rheological properties of polysaccharide solution was compared with commercial gums. Fig. 1 shows the viscosity of 1% polysaccharide solution at various shear rates. All the polysaccharide solutions showed high degree of pseudoplasticity that the apparent viscosity decreased with increase of shear rate. The viscosities could be expressed in the following equation (14);

$$\eta = K \cdot D^{n-1}$$

where η is apparent viscosity, K is consistency coefficient, n is flow behaviour index and D is shear rate. BT-4 polysaccharide solution showed pseudoplastic nature higher than xanthan and guar gum solution. The flow behaviour indices of 1% solutions of BT-4 polysaccharide, xanthan gum and guar gum were 0.09, 0.25 and 0.30, respectively. The high pseudoplasticity is very important in contributing good quality of food products (mouthefeel or flavor release) (15). The apparent viscosity of the microbial polysaccharide solution was compared with several commercial gums. The apparent viscosity of BT-4 polysaccharide solution was extremely high. The apparent viscosities of 1% solution of BT-4 polysaccharide, guar and xanthan gum were 1,769,512 and 466 mPa·s at 42 sec^{-1}. Especially, 1% BT-4 polysaccharide solution appeared almost gel-like when standing, but poured readily. As shown in this study it was concluded that polysaccharide produced by *Ps. delafieldii* BT-4 had competitiveness in viscosity. This suggested a possibility that it could be utilized as suspending, stabilizing agent or thickener in industry.

Concentration dependency of the apparent viscosity

The apparent viscosities at various concentrations of BT-4 polysaccharide as a function of shear rate are shown in Fig. 2. Viscosity increase was very significant below 0.5% of concentration as the concentration became higher and then constant increase was recognized. The apparent viscosities of 1.25, 1.0, 0.75, 0.5 and 0.25% solutions at the shear rate of 42 sec^{-1} were 2,681, 1,769, 914, 456 and 107 m Pa. s, respectively. The shear rate dependency was also increasing with the increase of concentration. The flow behaviour indices at the respective concentration were 0.07, 0.09, 0.18, 0.28 and 0.47 and the consistency coefficients were 85.64, 52.51, 19.22, 6.64 and 0.73 Pa. s respectively (Fig. 3). The viscosity changes could be expressed as a function of concentration in semilogarithmic or double logarithmic plotting as followig equation;

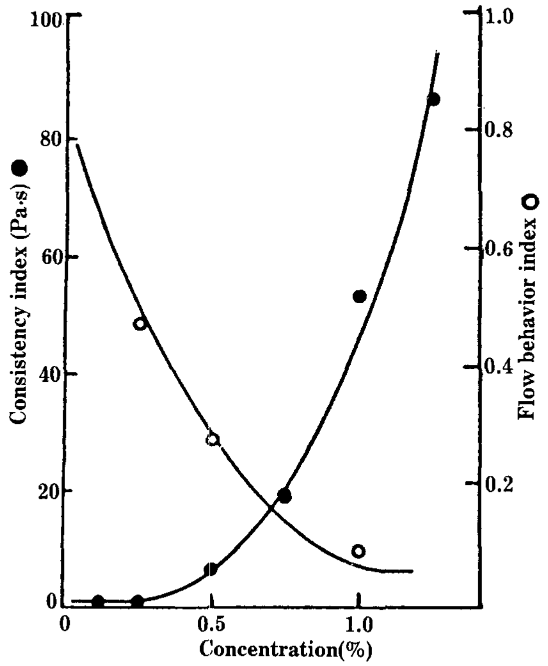

Fig. 3. Flow behaviour index and consistency coefficient of polysaccharide produced by *Pseudomonas delafieldii* BT-4 at various concentration.

$K = K_0 EXP\ (BC)$ (16)
$K = K_1 C^a$ (14, 17)

where K is consistency coefficient, K_0 and K_1 are intercept, B is concentration dependency factor and C is concentration. However, the double logarithmic plotting was able to be more closely correlated for the selected polysaccharide solution. The a values calculated by the least square fitting method were 3.60, 3.05, 3.13 and 2.87 and K_1 values were 55.22, 41.31, 26.02 and 12.76 Pa. s at 25, 45, 65 and 85°C. Therefore it was concluded that the concentration dependency of BT-4 polysaccharide solution was reduced as the temperature incerased. This kind of temperature dependency was reported on starch polymer (16) and microbial polysaccharide (17). The concentration dependency of the apparent viscosity of polysaccharide solution was found to be higher than xanthan gum solution (19). Speers and Tung (17) revealed that the dependency increased at the higher temperature.

pH dependency of apparent viscosity

A wide range of pH can be encountered in applying a new polysaccharide in food and other industrial products. Therefore, it is advisable to examine the stability in apparent viscosity of newly developed polysaccharide for understanding some basic properties useful for application.

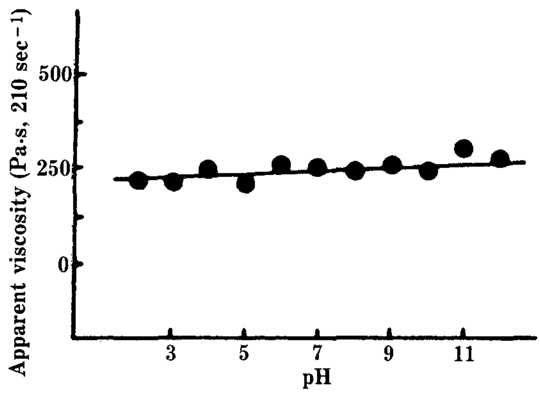

Fig. 4. Effect of pH on viscosity of 0.75% polysaccharide solution produced by *Pseudomonas delafieldii* BT-4.

Fig. 4. shows the apparent viscosities at 210 sec^{-1} at various pH of 0.75% BT-4 polysaccharide solution. The BT-4 polysaccharide solution had also virtual pH stability in apparent viscosity over a broad range of pH. The apparent viscosities of the solution at the pH of 3, 5, 7, 9 and 11 were 214, 209, 251, 261 and 298 mPa· s, respectively. Generally, most of microbial polysaccharide solution are reported to have pH dependency (18-20). They have a region where maximum viscosity can be achieved and above or below which the value decreased. Whereas, some polysaccharides produced by *Arthrobacter* sp. (21) and *Porodisculus pendulus* (14) were reported as extremely stable over a wide range of pH. A polysaccharide produced by *Alcaligenes* sp. (22) was found to be highly viscous at the pH between 6-10 and below 3, form gel, showing sharp increase in viscosity. In contrast, succinoglucan showed low viscosity at pH between 6-9 and below or above which it started to increase (20).

Effect of NaCl on the apparent viscosity

Various salts can cause viscosity change (18, 21-25), precipitation (15) and gelation (15) of polysaccharide solution. Xanthan gum was enhanced in thermal stability by the addition of NaCl (15). Many systems including foods contains certain level of electrolytes. Accordingly, it is useful to elucidate the possible effect of salt on the polysaccharide soultions.

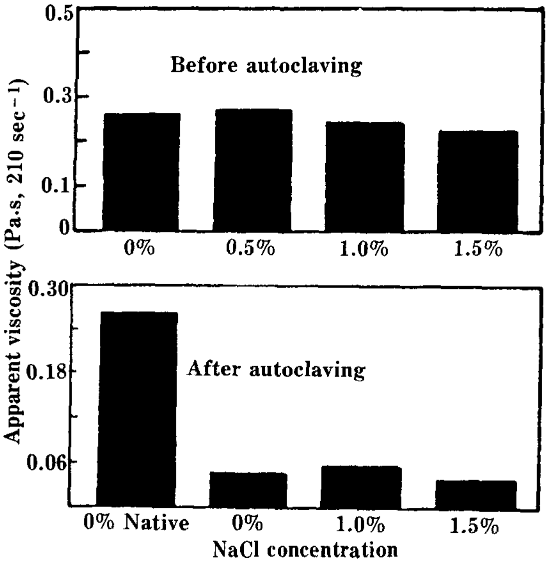

Fig. 5. Effect of NaCl on the viscosity of 0.75% polysaccharide solution produced by *Pseudomonas delafieldii* BT-4 before and after autoclaving at 121°C for 15 minutes.

Therefore NaCl was added into the polysaccharide solution and viscosity change was examined.

Fig. 5. shows the apparent viscosity of the 0.75% BT-4 polysaccharide solution as affected by NaCl concentration before and after autoclaving. There was no virtual change in apparent viscosity upon the addition of NaCl. It was not salt-precipitatable. The apparent viscosities of the solution containing 0, 0.5, 1.0 and 1.5% NaCl were 261, 270, 242 and 223 m Pa· s at the shear rate of 210 sec^{-1}. When autoclaving each solution at 121°C for 15 minutes, only less than 23% of original viscosity was remained and stabilizing effect on thermal reduction of apparent viscosity could not be achieved by adding NaCl. The polysaccharide solution was not thermally gellable.

Temperature dependency of the apparent viscosity

Many polysaccharide solution became less viscous as the temperature increased (17,21,26), whereas some microbial polysaccharides are stable over wide range of temperature (27) and some form gel upon heating (28-30). This kind of properties of polysaccharide solution is very important to get an insight for application. Therefore, the polysaccharide solution was subjected to temperature change and the rheological properties were examined.

Fig. 6. shows the viscosity-shear rate curve of 1%

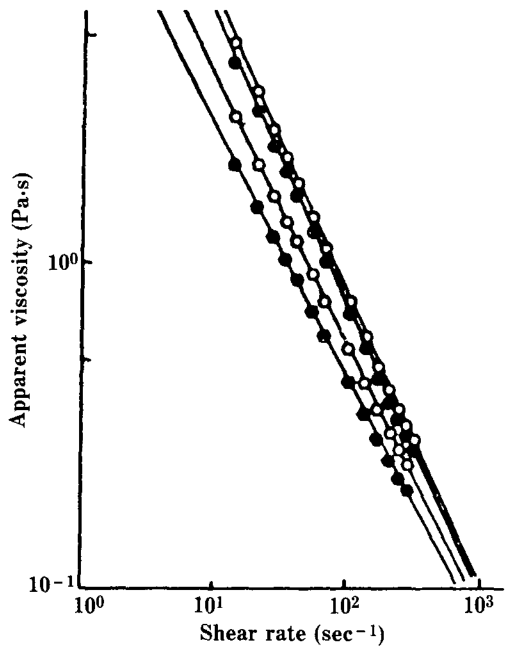

Fig. 6. Temperature dependency of 1% polysaccharide solution produced by *Pseudomonas delafieldii* BT-4.
(O: 25°C, ●: 45°C, O: 65°C, ●: 85°C)

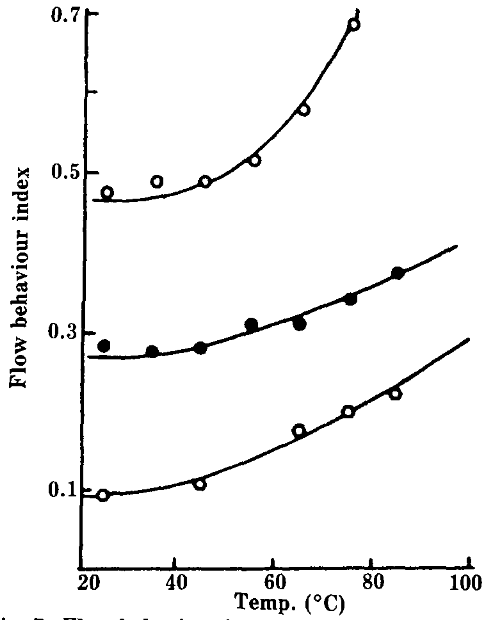

Fig. 7. Flow behaviour index of polysaccharide solution produced by *Pseudomonas delafieldii* BT-4.
(O: 1%, ●: 0.5%, O: 0.25%)

BT-4 polysaccharide solution at several temperatures. The apparent viscosities at 25, 45, 65 and 85°C were 1769, 1269, 922 and 89 m Pa· s, respectively at the sh-

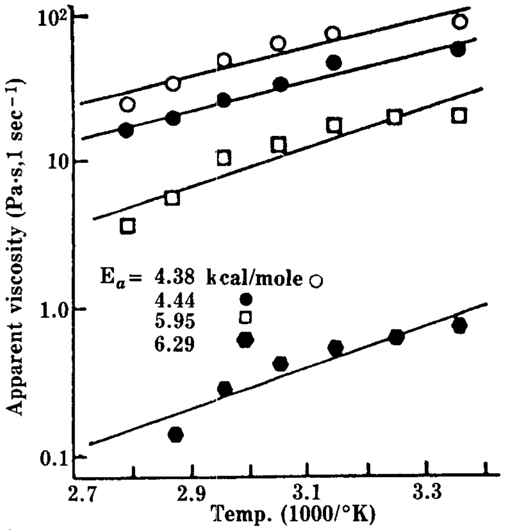

Fig. 8. Arrehenius plot of viscosity of polysaccharide solution produced by *Pseudomonas delafieldii* BT-4. (○: 1.25%, ●: 1.0%, □: 0.75%, ●: 0.25%)

ear rate of 42 sec^{-1}. The sensitivity of pseudoplasticity to the temperature of the polysaccharide solution was higher when the concentration was high (Fig. 7). The flow behaviour indices of 1.0, 0.5 and 0.25% solution were 0.09, 0.28 and 0.47 at 25°C, whereas they were 0.22, 0.37 and 0.69 at 85°C. Fig. 8. shows the plotting of consistency index versus the reciprocal of absoulte temperature. This curve could be expressed in Arrhenius relationship (31);

$$K = A \cdot EXP\ (-E_a/RT)$$

where K is consistency coefficient (Pa·s), A is frequency factor, E_a is activation energy of flow, R is gas constant (1.98 cal/mole-°K) and T is absolute temperature. The temperature dependency increased as the concentration of polymer solution became lower. The activation energy of flow of 1.25, 1.0, 0.75 and 0.25% BT-4 polysaccharide solution were 4.38, 4.44, 5.95 and 6.29 kcal/mole. They did not form gel upon heating and did not have inflection point on the viscosity-temperature curve. The curve was not sigmoid shape like xanthan gum (32). Tako *et al.* (18,32) found an inflection point on the curve of guar gum and the polysaccharide produced by coryneform bacterium and *Bacillus polymyxa*. They reported that there might be an alteration of three dimensional structure or dissociation of secondary binding in this inflection point, and that the activation energy increased with increasing the concentration.

요 약

*Pseudomonas delafieldii*의 배양액으로부터 분리한 다당류 용액의 물성을 검토하였다. 본 다당류는 매우 점도가 높은 의가소성 물질로 1% 용액의 겉보기 점도는 42 sec^{-1}에서 1769 mPa·s이었다. 이 용액은 pH에 대한 안정성은 있으나 열 안정성은 보이지 않았다. 이 용액의 유동 활성에너지는 4.44 kcal/mole이고, 농도의존성은 double logarithm으로 표현되었다.

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