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NOTE

Rheological Properties of a Novel High Viscosity Polysaccharide, A49-Pol, Produced by *Bacillus polymyxa*

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Abstract An exopolysaccharide, designated as A49-Pol, was produced from *Bacillus polymyxa* KCTC 8648P in nitrogen sufficient conditions. The viscosity of the culture broth increased up to 2×10^4 cP in 38 hours of culture and then decreased to 1.5×10^4 cP at 48 hours. The 1.0% (w/v) solution of purified A49-Pol represented pseudoplasticity with a viscosity of 2.7×10^4 cP which was two times higher than xanthan at the same concentration. The viscosity of the A49-Pol solution was also greatly affected by its concentration in comparison with the xanthan solution. The viscosity of 1.0% A49-Pol solution was 930-fold higher than its 0.2% solution, whereas the corresponding viscosities of xanthan solution experienced only 17-fold difference. The viscosity was observed to be maximum at pH 7.0 in both A49-Pol and xanthan solutions, and gradually decreased as the pHs of the polysaccharide solutions went to acidic or alkaline regions. The viscosity of A49-Pol solution was very sensitive to temperature compared to xanthan and decreased with increasing temperature. The viscosity of 0.6% solution of A49-Pol was 8,100 cP at 10℃ and 55 cP at 50℃. The viscosity was also affected by the presence of surfactants such as Span 20 and Triton X-100; with 0.5% Triton X-100 (v/v), the viscosity of A49-Pol solution increased by 50%.

Key words: Polysaccharide, xanthan, A49-Pol, viscosity, *Bacillus polymyxa*

Microorganisms are known to accumulate various kinds of polysaccharide inside or outside the cell [5, 11]. Many bacterial polysaccharides have characteristic rheological and physiological properties that are different from those of natural gums and synthetic polymers. These polysaccharides are also susceptible to biodegradation in nature and are environmentally less burdensome than synthetic polymers. Some microbial polysaccharides are produced on commercial scales and used for various purposes including food, medical, and industrial applications [18]; xanthan produced by *Xanthomonas campestris* [16, 17] and alginate by *Pseudomonas aeruginosa* [2] are used as food stabilizers, and dextran produced by *Leuconostoc mesenteroidses* [13] is used as a blood plasma extender. We isolated a bacterium from soil which produces an acidic extracellular polysaccharide. The bacterium is a spore forming, Gram-positive rod and was identified as *Bacillus polymyxa* [9]. The polysaccharide produced by the isolated *B. polymyxa* is water-soluble and aqueous solutions of the polysaccharide are highly viscous. This polysaccharide contains glucose, galactose, glucuronic acid, mannose and fucose, has an average molecular weight of 1.6 KDa and has been designated as A49-Pol. Viscosity is often the most important criterion in selecting a useful microbial polysaccharide since it gives some desirable properties to thickeners and emulsifiers [14]. Xanthan gum produced by *Xanthomonas campestris* has been widely exploited because it has unique rheology resulting from its high viscosity and stability [6, 10, 15]. In this paper, the rheological properties of A49-Pol, a novel polysaccharide produced by *B. polymyxa*, are described and compared with those of xanthan in a variety of facets.

The isolated organism was identified as *B. polymyxa*, deposited in the Korean collections for type cultures (Taejon, Korea) and given the collection number KCTC 8648P. The seed culture medium contained (per liter) 20 g glucose, 1 g $CaCO_3$, 1 g NH_4NO_3, 0.8 g K_2HPO_4, 0.6 g KH_2PO_4, 50 mg $MgSO_4\cdot7H_2O$, 50 mg $MnSO_4\cdot4\sim5H_2O$, 0.1 g yeast extract, 0.1 g tryptone, and 0.1 g soytone. The production medium had the same composition as the

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seed medium except for 60 g glucose, 4 g $CaCO_3$, and 2 g NH_4NO_3. Fermentation was carried out in a 5-*l* jar fermenter (Korea Fermenter Co., Incheon, Korea) equipped with a DO analyzer and pH controller. 100 ml of seed culture, cultivated at 30°C for 24 h in shake flasks, were transferred to the fermenter containing 1.9 *l* of the production medium.

The concentrations of cells and polysaccharide were determined by measuring the dry weights. An appropriately diluted sample was centrifuged at 15,000 rpm at 4°C for 30 min. The precipitated cells were collected, washed, recentrifuged, and then dried to a constant weight in a drying oven. The polysaccharide present in the supernatant was precipitated by adding three volumes of ethanol, recovered, and then dried to a constant weight. Glucose was determined by the dinitrosalicylic acid method [12]. Ammonia and nitrate were assayed by the indophenol method [1] and the enzymatic method using the Boeringher Manheim kit, respectively.

The exopolysaccharide described above was further purified by dissolving in 10% NaCl solution at 70°C, followed by dialysis and ethanol precipitation. The precipitated polysaccharide was then washed with ethanol three times and vacuum-dried. The purified polysaccharide was redissolved in distilled water. The viscosity of the crude culture broth and solutions of the purified polysaccharide were measured with a Brookfield Digital viscometer model DV-III with a small sample adapter (Brookfield Engineering Lab., U.S.A.). Measurements were performed at pH 7.0 and 25°C, and readings were taken after rotation for 2 min. The values obtained were averages of three measurements. To determine the effect of temperature on the viscosity of aqueous solutions of the polysaccharide, the viscosity was measured using a temperature-regulated small sample adapter. The temperature range tested was from 10–50°C; the system was allowed to equilibrate thermally between viscosity readings of the same sample. To determine the effect of pH on the viscosity, solutions ranging from pH 3–11 were prepared from a stock polysaccharide solution and mixed with sodium acetate/sodium phosphate stocks (100 mM each) titrated to the appropriate pH. The pH of the buffered polysaccharide solution was determined prior to the viscosity measurement.

Production of A49-Pol from *B. polymyxa*

The fermentative production of A49-Pol was studied. Cell growth, culture viscosity, pH and the amount of A49-Pol produced were monitored for 48 h (Fig. 1). Microbial polysaccharides are usually produced under nitrogen or phosphate limitation [3, 4, 7]. However, *B. polymyxa* KCTC 8648P continued to produce A49-Pol during cell growth under nitrogen sufficient conditions.

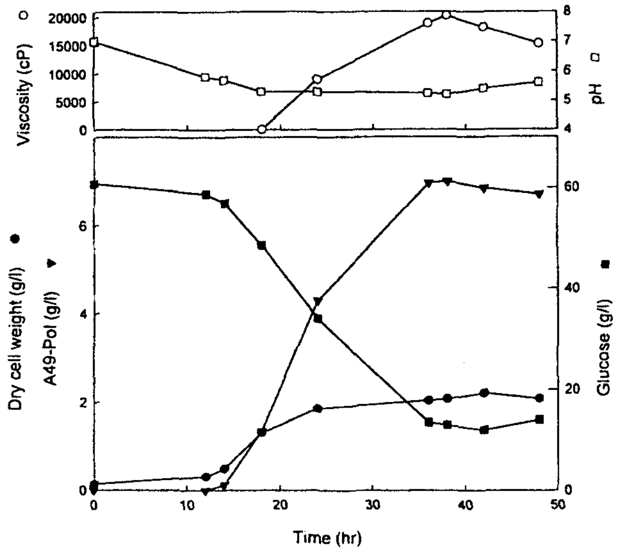

Fig. 1. A time course of fermentative production of A49-Pol, a water-soluble exopolysaccharide, by *B. polymyxa* KCTC 8648P.

Xanthan production by *Xanthomonas campestris* was also enhanced in higher nitrogen concentrations [15]. A final concentration of 7.0 g/l of A49-Pol was obtained; 4.3 g/l and 2.7 g/l of A49-Pol were produced during the cell growth and stationary stages, respectively. It was apparent that polysaccharide production by *B. polymyxa* KCTC 8648P was partially growth-associated. The A49-Pol concentration reached its maximum at 38 hours of cultivation, after which it remained relatively constant. The viscosity of the culture broth increased sharply to 2×10^4 cP in 38 hours of cultivation and then decreased to 1.5×10^4 cP in 48 hours. Because the concentration of A49-Pol remained constant, the decrease in the viscosity of the culture broth suggests that structural change(s) may have occurred during this stage. Ammonia and nitrate, nitrogen sources, were not depleted during the cultivation of the culture (data not shown here). The cell concentration was at a relatively low level even under the nutrient-sufficient condition (2.1 g/l). There was a slight decrease in the pH of the culture broth during the course of fermentation.

Rheological Properties of A49-Pol

Most of the commercial applications of microbial polysaccharides depend on their rheological properties. Xanthan is the most widely used microbial polysaccharide and has a variety of applications in both food and pharmaceutical industries. Rheological properties of A49-Pol were compared with those of xanthan (produced by *Xanthomonas campestris*, Sigma Co.). The effect of shear rate on solution viscosity was examined. As shown in Fig. 2A, viscosities of both polysaccharides showing

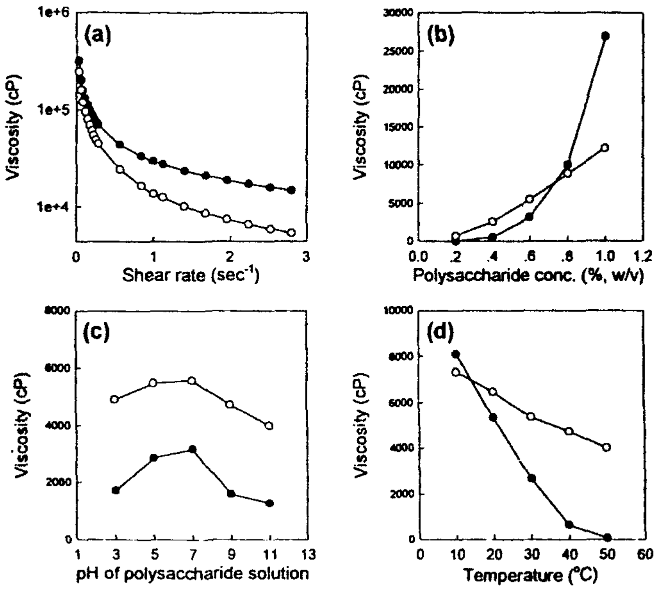

Fig. 2. Effect of shear rate (a), polysaccharide concentration (b), pH (c), and temperature (d) on the viscosity of A49-Pol (●) and xanthan (○) solutions.

In (b), (c), and (d), viscosities were measured at a shear rate of 1 sec^{-1} with polysaccharide solutions of 0.6% (w/v). In (a), 1.0% (w/v) solution was used for the measurement of viscosity.

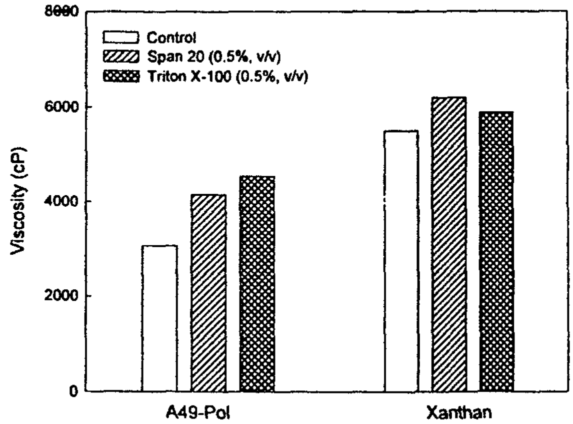

Fig. 3. Effect of surfactants on viscosity of 0.6% (w/v) solution of A49-Pol and xanthan at a shear rate, 1 sec^{-1}.

pseudoplastic behavior decreased with increasing shear rate. A49-Pol solution showed a lower degree of pseudoplasticity than xanthan. This suggests that the viscosity of A49-Pol solution was less affected by changes in shear rate than xanthan. Figure 2B shows the change in viscosity of different concentrations of polysaccharides at a shear rate of 1 sec^{-1}. The viscosity of A49-Pol solution increased more drastically than xanthan as their concentrations increased. The viscosity of 1.0% (w/v) solution of A49-Pol was 930-fold higher than its 0.2% solution, whereas the corresponding viscosities of xanthan solution experienced only a 19-fold difference. At the concentration of 1.0% (w/v), A49-Pol solution was highly viscous. The viscosity of 1.0% A49-Pol solution was 2.7×10^4 cP, which was two times higher than 1.0% xanthan solution. The effects of temperature and pH on the solutions viscosity were investigated. Figure 2C shows that the viscosities of the solutions of A49-Pol and xanthan change in the pH range tested, and the highest viscosity was observed at pH 7. The pH stability of the viscosity of A49-Pol solution was similar to that of xanthan. Figure 2D shows that the viscosity of A49-Pol solution decreased sharply with increasing temperature, while xanthan did so to a much lesser degree. The viscosity of 0.6% (w/v) A49-Pol solution was 8,100 cP at 10°C and 55 cP at 50°C. Viscous behavior of the solution was not further observed at temperatures higher than 50°C. The viscosity of succinoglycan solutions have also been reported to be temperature dependent, with the viscosity approaching that of water at 60°C [8]. Other rheological studies indicated that the viscosities of A49-Pol and xanthan solutions increase with the addition of surfactants (0.5% (v/v) of Triton X-100 or Span 20) (Fig. 3). The viscosity of A49-Pol solution increased by 50% (from 3,000 cP to 4,500 cP) in the presence of 0.5% Triton X-100. In a previous study, we observed that both the production of A49-Pol and the viscosity of culture broth were significantly enhanced with the addition of 0.5% Triton X-100 during the cultivation of the culture. It may be suggested that the conformation of A49-Pol in solution changes in the presence of the surfactants. Among all the above-mentioned rheological characteristics of A49-Pol, it was most strongly noted that the viscosity of A49-Pol solution is affected by the incubation temperature and its concentration. This also indicates that the desired viscosity of A49-Pol solution can be easily obtained by changing the temperature or A49-Pol concentration. These characteristics would provide selective advantages over xanthan in certain commercial applications. The high viscosity of A49-Pol at low temperatures could improve such qualities of frozen foods as texture, mouth-feel, and flavor release by its incorporation in small amounts. The higher viscosity of A49-Pol than xanthan at 1% concentration would make it more suitable than xanthan for use as a lubricant for drilling mud which requires high viscosity. Considering that the high viscosity of the culture broth, causing a problem for mass transfer, is the most critical constraint in the large scale production of the polysaccharide [11], a slight increase of culture temperature in A49-Pol production might significantly enhance the titer of polysaccharide produced. Further investigations are underway to improve the yield of A49-Pol by optimizing fermentation conditions including such physical parameters as temperature.

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