

Heteropolysaccharide Formation by *Arthrobacter viscosus* Grown on Xylose and Xylose Oligosaccharides

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***Arthrobacter viscosus* NRRL B-1973 produces its viscous extracellular polysaccharides (EPS) when grown on media containing xylose or enzymatic xylan hydrolysates. Crude EPS formation from xylose averaged 12 g/liter when initial culture pH was adjusted to 8.0 and total nitrogen was limited to 0.03%. Purified EPS from pentose and hexose substrates were analyzed for their monosaccharide, acetyl, and uronic acid components, intrinsic viscosities, and average molecular masses. Differences were apparent in degrees of acetylation, molecular masses, and intrinsic viscosities of the heteropolysaccharides produced on different carbon sources.**

Although much effort has been expended on bioconversion of plant carbohydrates to commercial solvents, relatively little attention has been devoted to alternative end-use products. Hemicellulose extracted from wood provides an enormous reservoir of fermentable carbon which could be integrated into comprehensive schemes for the production of diverse useful products. Microbial extracellular polysaccharides (EPS) represent a class of high-value polymers with many industrial applications. Because of their unique rheological properties, they have been used as emulsifiers, as stabilizers, and as texture enhancers in the food industry (14, 25, 32). Additional applications have been as adjuncts to oil well drilling and as viscosifiers in the cosmetic, textile, and pharmaceutical industries (32, 33). Specifically, EPS such as dextran and xanthan have grown in commercial significance in the pharmaceutical and food industries.

This report describes a project which was initiated to produce and characterize high-value polysaccharides from hemicellulose. The nonpathogenic soil bacterium *Arthrobacter viscosus* NRRL B-1973, earlier shown (4, 8) to form an EPS containing glucose, galactose, and mannuronic acid in equal proportions from hexoses, was chosen for further examination regarding the formation of such biopolymers from xylose and xylose oligomers. In this work, a comparison is made between the glucose- and xylose-derived polysaccharides in terms of yield, degree of substitution, and rheological characterizations.

MATERIALS AND METHODS

Culture conditions. *A. viscosus* NRRL B-1973 was obtained from the American Type Culture Collection (ATCC 19584; Rockville, Md.). Cultures were maintained on nutrient agar (Difco) slants and transferred monthly. A modified Haggström (10) liquid growth medium was used for polysaccharide production and contained the following (in grams per liter): peptone, 1.0; yeast extract, 1.0; NH_4Cl , 0.1; Na_2HPO_4 , 0.6; KH_2PO_4 , 0.4; and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2. Trace elements were added to the following final concentration (micromolar): $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.036; H_3BO_3 , 0.097; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.017; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.08; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.019; and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.008. The culture medium was brought to a pH of 8.5 with 10 N KOH and sterilized by autoclaving

separately, prior to the addition of a carbon source. Carbon sources were added aseptically to a final concentration of 3.0% (wt/vol). Liquid cultures were inoculated with 72-h *A. viscosus* NRRL B-1973 starter cultures to a final concentration of 0.2% (wt/vol). These cultures were then incubated at 28°C with shaking at 150 rpm.

Carbon sources. Reagent-grade glucose, xylose, and xylan (from oat spelt) were obtained from Sigma Chemical Co. (St. Louis, Mo.). The xylan was enzymatically hydrolyzed to a mixture of xylosides by using a partially purified enzyme preparation (22-24) from *Trichoderma longibrachiatum* culture (obtained from C. J. Wang, College of Environmental Science and Forestry, Syracuse, N.Y.). Crude enzyme (250 μl containing 10 U [24]) was added to a 5% (wt/vol) xylan suspension prepared in 0.05 M sodium citrate (pH 4.8). The suspension was then stirred for 24 h at 50°C. The slurry was centrifuged at $4,000 \times g$ for 15 min to remove unreacted xylan, and the clear yellow xylan hydrolysate was filtered through a 0.22- μm -pore-size filter (Millipore Corp., Bedford, Mass.) and lyophilized. Reducing sugar content was determined by the method of Miller (18).

EPS extraction. Shake flask cultures (100 ml) were diluted 1:2 with distilled H_2O and centrifuged at $27,000 \times g$ for 30 min. Hexadecyltrimethylammonium bromide (Eastman Kodak Co., Rochester, N.Y.) was added to the supernatants to a final concentration of 0.175% (wt/vol) (19). The gelatinous polysaccharide precipitate was collected by centrifugation at $27,000 \times g$ for 30 min. The pellet, considered as crude EPS, was air dried and weighed. The polysaccharide pellet was resuspended in 2 M NaCl, precipitated with 3 volumes of 95% ethanol, and dialyzed against deionized H_2O to remove bound quaternary ammonium salt. Protein and DNA were determined by the methods of Lowry et al. (16) and Burton (3), respectively.

Acid hydrolysis of polysaccharides. Five milligrams of each purified polysaccharide was suspended in 0.5 ml of 2 M trifluoroacetic acid (Aldrich Chemical Co., Inc., Milwaukee, Wis.) and hydrolyzed for 4 h at 100°C in a heating block. Samples were then dried under a stream of nitrogen gas and reconstituted in 0.5 ml of deionized H_2O . Component sugars were analyzed by thin-layer and high-pressure chromatography.

Thin-layer chromatography. Hydrolyzed polysaccharides at a concentration of 10 mg/ml were run (5 μl) on Whatman K5 silica gel glass plates (21), developed with an *n*-butanol-

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pyridine-water (6:4:3) solvent, and visualized by 3% (wt/vol) *p*-anisidine hydrochloride (Sigma) prepared in *n*-butanol (31). The thin-layer chromatography plates were sprayed evenly, air dried, and heated at 100°C for 10 min.

High-performance liquid chromatography. Filtered 20- μ l EPS hydrolysates (10 mg/ml) were analyzed on a Perkin-Elmer series 10 liquid chromatograph (Norwalk, Conn.) by using an Aminex HPX-87P (Bio-Rad, Richmond, Calif.) column (300 by 7.8 mm) at 85°C with Carbo-P microguard cartridges. Elution was effected with glass-distilled H₂O at 0.6 ml/min. Polysaccharide components were detected with a Perkin-Elmer LC-75 spectrophotometric variable wavelength detector (192 nm) or a Perkin-Elmer LC-25 refractive index detector. Monosaccharide quantitations were obtained with a Hewlett-Packard model 3394A peak integrator (Avondale, Pa.).

Gel permeation chromatography. Polysaccharide molecular masses were determined by using methods previously described (20). Samples (0.5 ml) containing 2 mg of polysaccharide were placed on a glass Econo-column (Bio-Rad) (1.5 by 100 cm) packed with Sephacryl S-400-HR (Sigma) and developed with morpholinepropanesulfonic acid (MOPS) buffer (Research Organics, Inc., Cleveland, Ohio) (20). Fifty-drop fractions (1.3 ml) were collected by using a Gilson model FC-80K fractionator (Middleton, Wis.) under a pressure head from a 500-ml reservoir. Fractions were analyzed for total carbohydrate by the phenol-sulfuric method (7). Dextran standards used were approximately 6,000, 15,000 to 20,000, 40,000, and 70,000 (Fluka Chemical Corp., Ronkonkoma, N.Y.) and 170,000, 600,000, and 2,000,000 (Polysciences Inc., Warrington, Pa.) Da.

Intrinsic viscosity. Polysaccharides at an initial concentration of 0.25% were solubilized in 0.15 M NaCl. Viscosity was measured over four successive dilutions of this initial concentration at 25°C by using a Cannon-Ubbelohde type viscometer size 75 (Cannon Instrument Co., State College, Pa.). Intrinsic viscosity measurements were calculated as previously described (6).

Apparent viscosity. The effects of polymer concentration, shear thinning, and salt on EPS viscosity were measured by using a Brookfield digital viscometer model DV-II at 25°C (Brookfield, Mass.).

Mannuronic acid analysis. The hexuronic acid content of purified EPS was determined by the *meta*-hydroxydiphenyl method (2).

Acetyl group assay. The degree of polysaccharide acetylation was determined by using the hydroxamic acid reaction (17).

Polyacrylamide gel electrophoresis. Fifty micrograms of EPS was added to each tube gel containing 10% polyacrylamide. Buffer consisting of 0.005 M Tris-glycine (pH 8.3) was used with a constant current of 1 to 2 mA per tube and a maximum of 5 tubes per run for 40 to 50 min (26). Polysaccharides were visualized with toluidine blue reagent (26). The Coomassie stain was used to evaluate the presence of contaminating protein.

RESULTS

The relationship between cell growth of *A. viscosus* and product formation measured as the dry weight of crude acidic polysaccharide is shown in Fig. 1a and b. Polysaccharide production reached a maximum of 12 g of crude product per liter of growth medium after 4 days of growth with a concomitant decrease in culture pH and viable cell count. In cultures grown on xylose-containing medium, cells entered

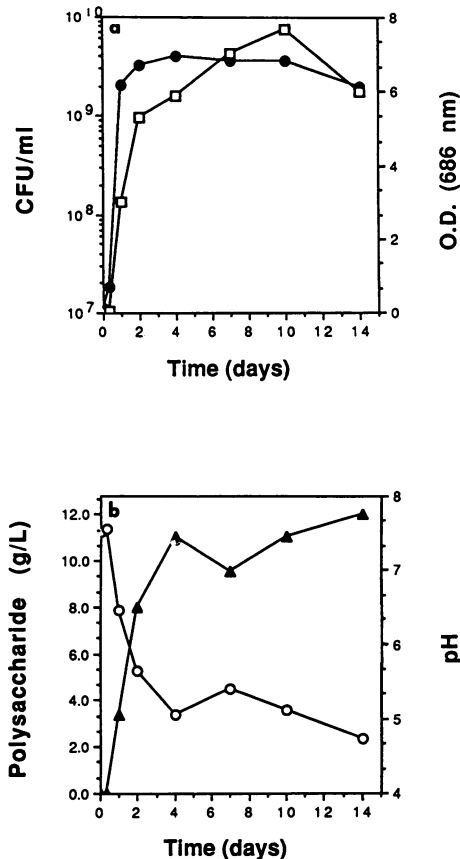


FIG. 1. Culture characteristics of *A. viscosus* NRRL B-1973 optimized for polysaccharide production on xylose-containing medium. (a) Culture viability and absorbance over time. Symbols: ●, CFU/ml; □, optical density (O.D.; 686 nm). (b) Polysaccharide production and culture pH over time. Symbols: ▲, EPS; ○, pH.

stationary phase after 2 days of incubation. Shake cultures incubated at 28°C exhibited the highest levels of product formation, while stationary cultures at the same temperature demonstrated relatively poor cell growth and lowered concentrations of EPS. Previous studies (4, 25) have indicated a similar dependence on aeration for optimal product formation.

Influence of pH on polysaccharide production. The adjustment of culture pH prior to inoculation with *A. viscosus* NRRL B-1973 resulted in the polysaccharide production profiles obtained in Fig. 1b. When adjusted to an initial pH of 8.0, *A. viscosus* produced the highest concentration of polysaccharide, approaching 17 g of crude product per liter after two days of growth. During this time, culture pH was allowed to drop freely, approaching a final value of approximately 4.0 (Fig. 1b). Previous studies with glucose-grown *A. viscosus* NRRL B-1973 EPS had shown that maximal culture growth occurred at pH 6.1 (4, 8). In our study an initial culture pH of neutral (6.8), alkaline (9.0), or slightly acidic (6.0) produced successively lower yields of EPS, respectively.

A bimodal polysaccharide production profile is demonstrated in Fig. 2 for a culture of *A. viscosus* with an initial pH of 8.0. The possibility that as acidic products accumulate a subsequent lowering in culture pH produces a growth-inhibited decrease in EPS was tested. Buffer was added to

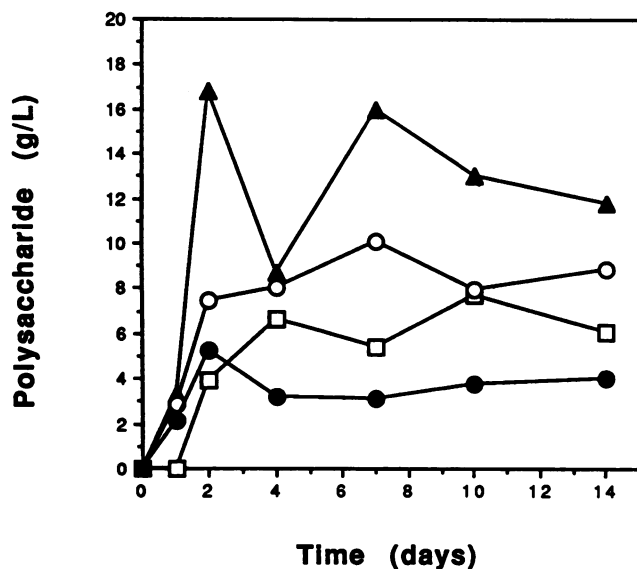


FIG. 2. Influence of initial pH on polysaccharide production from xylose-grown cultures of *A. viscosus* NRRL B-1973. Symbols: ●, pH 6.0; ○, pH 6.8; ▲, pH 8.0; □, pH 9.0.

the initial culture medium to maintain pH and increase both growth and EPS production. Since Tris-HCl interfered with cell metabolism (results not shown), a mono- and dibasic phosphate buffer was examined (Fig. 3) and a concentration of 10 mM was found adequate to maintain culture pH above 5.0 for 14 days. As the buffering capacity of the culture was increased beyond this concentration, polysaccharide production decreased (Fig. 3).

Effect of nitrogen limitation on EPS production. The culture medium C:N ratio, given as the total carbon divided by the total nitrogen and carbon added to the growth medium, is depicted in Fig. 4. This ratio is represented as such in order to produce a finite range (0.0 to 1.0) over which the amount of carbon and nitrogen could be varied. As nitrogen (in the form of NH_4Cl) was limited to 0.1 g/liter, EPS production increased. Further nitrogen depletion resulted in a decrease in EPS production. A C/C+N value of 0.988 corresponded to

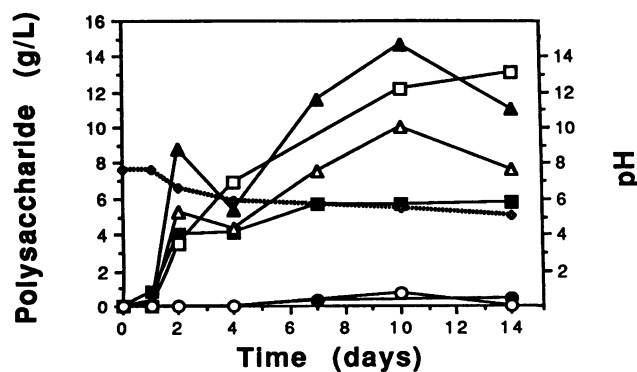


FIG. 3. Polysaccharide production as a function of phosphate concentration over time in nitrogen-limited *A. viscosus* NRRL B-1973 cultures. Phosphate buffer was added to the following final concentrations (millimolar): 0 (■), 2 (△), 5 (▲), 10 (□), 20 (●), and 25 (○). ◇, culture pH over time of the culture in 10 mM phosphate buffer.

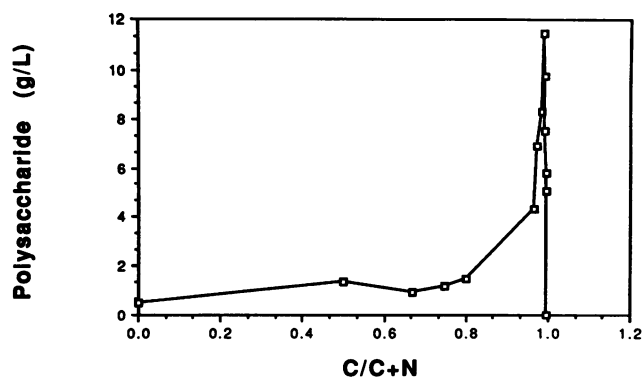


FIG. 4. Effects of nitrogen limitation on polysaccharide production by *A. viscosus* NRRL B-1973. □, EPS from xylose-containing medium.

the optimum crude EPS production value of 11.48 g/liter (Fig. 4). Total nitrogen in the medium was calculated to be 0.033% at this EPS optimum. Yeast extract and peptone were also varied in the medium in earlier studies with no correlation to EPS production (data not shown).

Carbon source optimization. Prior to N optimization, xylose, at a concentration of 3% (wt/vol), was found to produce an optimum EPS value of 4.43 g/liter. Also, the culture pH decreased from 8.0 to 4.0 during the course of the fermentation. EPS production in the nitrogen-limited medium more than doubled at concentrations of 3% (wt/vol) xylose. Despite the increased amounts of acidic EPS produced, a culture pH profile which was similar to that in Fig. 3 resulted. Therefore, culture pH was not a function of polysaccharide concentration.

Compositional analysis of *A. viscosus* from different carbon sources. The glucose-, xylose-, and xylan hydrolysate-produced polysaccharides were acid hydrolyzed, and component sugars were qualitatively identified by thin-layer chromatography. Three carbohydrate components were present in all polysaccharides regardless of carbon source. These constituents corresponded to R_f values of 0.720, 0.580, and 0.505, which provisionally indicated the presence of manuronic acid, glucose, and galactose, respectively.

High-pressure liquid chromatography analysis of the hydrolyzed *A. viscosus* polysaccharides was used to quantify the constituent sugars. For polysaccharides produced on glucose, xylose, and a xylan hydrolysate, the glucose and galactose components were found to be present in equimolar amounts (Table 1). These data are consistent with previously reported results for the glucose-derived *A. viscosus* polysaccharide (8, 12, 27). *A. viscosus* polysaccharides were also analyzed by using the *meta*-hydroxydiphenyl assay (2) for manuronic acid content. All *A. viscosus* polysaccharides tested were found to contain equivalent manuronic acid by weight (Table 1). *A. viscosus* polysaccharide produced from glucose has been reported to contain 25% manuronic acid (12), although our glucose-grown polysaccharide contained 18% manuronic acid.

The percentage of acetyl by weight was found to be 29.3% in the xylose-produced polysaccharide and 20.0% in the xylan hydrolysate-produced *A. viscosus* polysaccharide (Table 1). A value of 24.4% acetyl was determined in glucose-produced *A. viscosus* NRRL B-1973 EPS and coincides with the previously reported value of 25% (8, 12).

Molecular mass determinations. A series of molecular mass

TABLE 1. Characterization of *A. viscosus* NRRL B-1973 polysaccharides

C source	% Glucose	% Galactose	% Mannuronic acid ^a	% Acetyl ^b	% Total polymer weight	Avg molecular mass ^c (kDa)	[η] ^d
Glucose	28.7 \pm 0.7	30.0 \pm 2.5	18.0 \pm 0.7	24.4 \pm 0.8	101.1 \pm 4.7	900	19.42
Xylose	30.0 \pm 2.5	31.0 \pm 2.1	17.3 \pm 1.3	29.3 \pm 1.9	107.6 \pm 7.8	1,500	25.35
Xylan hydrolysate	25.0 \pm 2.9	30.7 \pm 3.2	17.9 \pm 0.7	20.0 \pm 0.5	93.6 \pm 7.3	600	12.63

^a *meta*-hydroxydiphenyl method (2).

^b Hydroxamic acid reaction (17).

^c Gel permeation chromatography (20).

^d Intrinsic viscosity determined by using 0.15 M NaCl at 25.0°C.

standards ranging from 6 to 2,000 kDa was run on a gel permeation column (20), and Fig. 5 illustrates the resultant standard curve obtained when eluted column fractions containing dextrans of known molecular masses were plotted against the corresponding molecular masses. The molecular masses of *Arthrobacter* polysaccharides were reproducibly determined to be 900 kDa when grown on glucose, 1,500 kDa for xylose, and 600 kDa for the xylan hydrolysate.

Polysaccharide intrinsic viscosities. High intrinsic viscosity values, obtained for each polysaccharide, were correlated with molecular mass values (Table 1). For example, the lowest viscosity was observed for the xylan hydrolysate-produced EPS, which was also determined to have the lowest molecular mass (600 kDa).

EPS rheology. EPS from glucose-, xylose-, and xylan hydrolysate-containing media as well as a commercially available xanthan comparison standard demonstrated shear-thinning behavior characteristic of pseudoplastic solutions (Fig. 6a). The viscosities of glucose- and xylan hydrolysate-produced EPS were temperature stable over a 20 to 80°C range (Fig. 6b). In the presence of increasing NaCl concentrations the glucose-produced EPS increased in apparent viscosity to a maximum of 1.0% NaCl and then decreased (Fig. 6c). All other EPS polymers maintained relatively constant viscosity in the presence of increasing salt.

DISCUSSION

A. viscosus NRRL B-1973 acidic EPS produced from cultures grown on pentoses were isolated and compared with the previously characterized EPS (4, 8, 12, 27) from glucose. While polysaccharide production was dependent upon cell growth, it was also possible to obtain dense cell growth without reaching maximum EPS levels (15). Culture optimizations produced EPS values exceeding 10.0 g/liter while maintaining dense cell growth. Initial culture pH values higher or lower than an optimal value of 8.0 produced lower values of crude EPS. This phenomenon may be explained by the observation that cultures inoculated into medium of low pH more rapidly attained a pH value restrictive to cell viability. A slightly basic growth medium pH of 8.0 reduced cell growth efficiency but allowed EPS to accumulate at an increased rate prior to the development of the restrictive pH level of 5.0. These results are in contrast to an earlier optimization study utilizing glucose in which a pH range of 6.8 to 7.2 was shown to produce the highest yields of EPS (4).

Attempts to maintain culture pH above a critical level did not result in increased yields of EPS. The addition of Tris-HCl buffer to the medium inhibited EPS production. *A. viscosus* responded to phosphate concentrations of 5 to 10 mM by producing near maximum levels (12 to 15 g/liter) of EPS, despite a lack of buffering capacity at these concentra-

tions. Higher concentrations of phosphate were effective at maintaining culture pH near 8.0 but completely inhibited polysaccharide production (Fig. 3). A critical level of phosphate is required for the synthesis of phosphorylated sugar nucleotides as precursors to EPS biosynthesis, and this need may take precedence over maintaining pH near neutrality.

Yields for the xylose- and xylan hydrolysate-produced *A. viscosus* polysaccharides were lower compared with those of the glucose-produced EPS. In general, xylose is not as universally metabolizable as glucose since isomerization and transketolation are required prior to further utilization (9). The additional enzymatic interconversions and carbon allocation may account for the observed yield differences in EPS production from different carbon sources. Xanthan gum, currently produced commercially by a new method, represents a 60% conversion of substrate glucose (25), while microbial alginate yields approach 50% bioconversions from sucrose or glucose (14). In this work, we now demonstrate that *A. viscosus* can form its EPS in some 30 to 50% yield from various types of xylan hydrolysates.

Nitrogen limitation resulted in the single largest polysaccharide production increase. Limiting the nitrogen concentration to 0.1 g of NH₄Cl per liter increased EPS production to 11.48 g/liter. This corresponded to a C/C+N value of 0.988. A similar response has been previously reported for

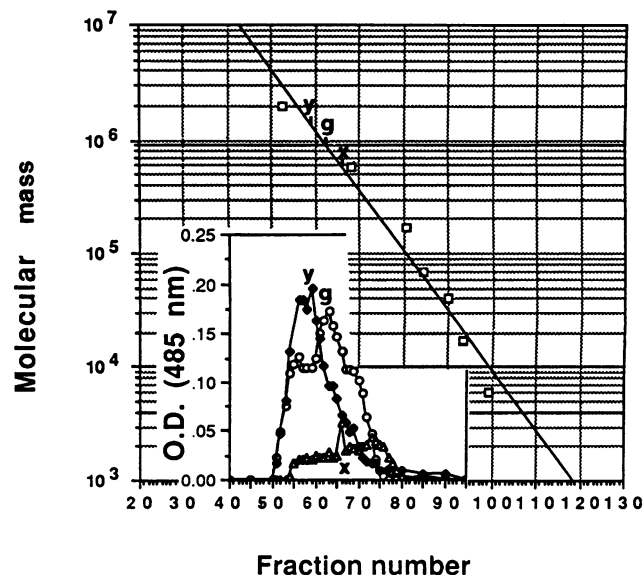


FIG. 5. Molecular mass determinations for *A. viscosus* NRRL B-1973 polysaccharides by gel permeation chromatography. Symbols: \square , dextran standards; g, glucose-derived EPS; y, xylose-derived EPS; x, xylan hydrolysate-derived EPS.

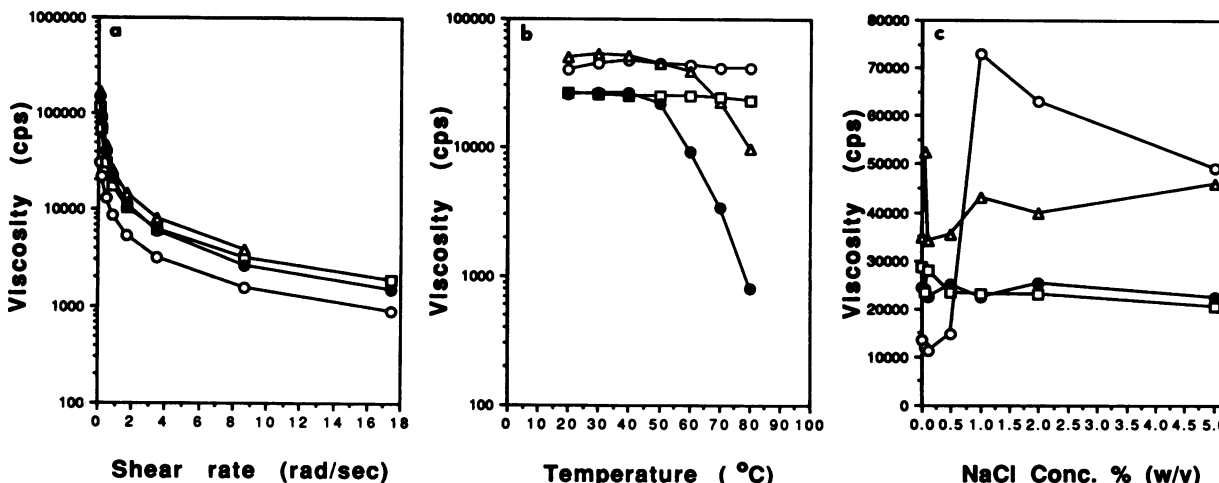


FIG. 6. Rheological comparison of shear thinning (a), the effects of temperature (b), and salt concentrations on the apparent viscosities of 1.0% (wt/vol) solutions of EPS in distilled water (c). Viscosity measurements in panels b and c were determined at a constant shear rate of 0.44 rad/s. Symbols: ○, EPS from glucose-containing medium; □, EPS from xylan hydrolysate-containing medium; △, EPS from xylose-containing medium; ●, xanthan comparison standard.

the glucose-produced polysaccharide from *A. viscosus* (4). Previous studies by Sutherland (28) indicate that there may be a system of priorities within bacterial cells ensuring that peptidoglycan synthesis occurs; next comes lipopolysaccharide, and finally exopolysaccharide. Considering this possibility, restricting nitrogen to a critical level may prevent utilization of carbon for supplementary amino acids, peptides, or peptidoglycan. This may result in a greater proportion of available carbon being converted directly to EPS.

Use of xylose as a carbon source at a concentration of 3% (wt/vol) produced an optimum EPS value of 4.43 g/liter after 3 to 4 days of incubation. The same xylose concentration resulted in 9.75 to 11.50 g of *A. viscosus* polysaccharide per liter in nitrogen-limited growth medium.

The homogeneity of the purified EPS from different sources was tested by using polyacrylamide gel electrophoresis (unpublished data). A single polysaccharide band was visualized in each case by using toluidine blue reagent, and absence of protein was confirmed by using the Coomassie blue stain. Protein and DNA contents were found to compose less than 6% by weight for all polysaccharides tested.

The purified EPS produced from *A. viscosus* NRRL B-1973 cultures grown on glucose-, xylose-, and xylan hydrolysate-containing medium were all found to contain identical carbohydrate constituents: glucose, galactose, and mannuronic acid. Previous work with *Xanthomonas campestris* has shown that the carbohydrate composition of polysaccharide produced from various carbon sources is usually constant (29, 30), while acetyl and other substituent groups may vary (30).

Similarly, the glucose-produced EPS from *A. viscosus* was previously shown to contain glucose, galactose, and mannuronic acid in equimolar ratios (4, 8, 12, 27). By using the *meta*-hydroxydiphenyl method (2), mannuronic acid content was assayed to be 18.0, 17.3, and 17.9% in the glucose-, xylose-, and xylan hydrolysate-produced polysaccharides, respectively (Table 1). These values are lower than the 25% value originally reported (8, 12, 27) for *A. viscosus* NRRL B-1973 EPS. Water contamination of the hygroscopic polysaccharide may account for this difference, even though all samples were lyophilized to a constant weight prior to

chemical analyses (30). As shown in Table 1, total polymer weights are accountable despite the slightly lower mannuronic acid values. In addition, differences from mannuronic acid values in the original study may be due to the presence of hexoses which interfere with assay specificity (2); higher concentrations of tetraborate, which is known to severely affect the absorbance of the chromogen 5-formylfuroic acid (13); and lower assay temperatures (55°C), which result in slightly higher absorbance for samples containing uronic acids (13).

The acetyl content for these polymers produced from medium containing glucose, xylose, or the xylan hydrolysate was found to be 24.4, 29.3, and 20%, respectively (Table 1). The acetyl value for glucose-derived EPS is consistent with previously published results (8, 12, 27). Quantitative differences in acetyl content resulting from different carbon sources may be expected. It has been shown with EPS from *X. campestris* that the degree of acetylation may be dependent on the substrate used as well as the particular stage of growth in batch culture (29).

Size variations among the *A. viscosus* polysaccharides were positively correlated with their intrinsic viscosities. The intrinsic viscosities were determined to be 19.25, 25.27, and 8.63 for the EPS from glucose-, xylose-, and xylan hydrolysate-grown cultures, respectively (Table 1). The corresponding average molecular mass values were 900, 1,500, and 600 kDa, respectively (Table 1). It is interesting to note that acetyl content, intrinsic viscosity, and average molecular mass show a similar correlation. Polysaccharide viscosity has previously been shown to be dependent upon molecular mass, polymer conformation, and intermolecular interactions (11).

The relationship between acetyl content and solution viscosity or molecular mass has generated controversy. It has been shown that a high degree of acetylation interferes with polysaccharide solubility, thus affecting intermolecular interactions (12). In another study, interactions between molecules could be substantially altered upon deacetylation of polysaccharides without altering the basic conformation of the native polymers (1). In concentrated EPS solutions above 0.05% (wt/vol), acetate was shown to decrease vis-

cosity (11). By using lower concentrations of polymer (0.02%) (wt/vol) it was shown that acetyl content had no influence either on xanthan dilute solution viscosity or on its intrinsic viscosity at a given molecular mass (5). Only molecular chain length appears to be related to viscosity in dilute solutions. It was concluded that at high concentrations of solute, the EPS intermolecular interactions interfere with the true viscosity relationships of the polymer.

Each of the *A. viscosus* NRRL B-1973 polysaccharides produced on either hexose or pentose demonstrated similar apparent viscosities and characteristic shear-thinning rheological characteristics (Fig. 6a) exhibited by pseudoplastics such as paints, emulsions, and other dispersions. The glucose- and xylan hydrolysate-produced EPS maintained stable apparent viscosities over a 20 to 80°C temperature range (Fig. 6b). However, the xylose-produced EPS and xanthan gum decreased in viscosity at temperatures above 50°C (Fig. 6b). The polymers were all stable to a pH range of 4 to 10. The *Arthrobacter* polymer is gel-like and exhibits rheological properties similar to those detailed for xanthan gum. The rheological stability of the *Arthrobacter* EPS to temperature, pH, shear rate, or salt concentration may offer selective advantages over the use of commercially available xanthan.

The unusual increase in apparent viscosity of the glucose-produced EPS in the presence of salt indicates their potential applications as lubricants or rheological stabilizers in saline environments (Fig. 6c). However, the EPS component(s) responsible for rheological variations still needs to be identified. In addition to further rheological characterizations of these extracellular polysaccharides, future work will involve an analysis of the enzymatic synthesis of these polymers in concert with structural differences obtained in mutants deficient in polysaccharide production.

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REFERENCES

- Atkins, E. 1987. Effect of mono-*o*-acetyl groups on the conformation and interactions of microbial polysaccharides, p. 177-186. In M. Yalpani (ed.), *Industrial polysaccharides: genetic engineering, structure/property relations and applications*. Elsevier Science Publishers B.V., Amsterdam.
- Blumenkrantz, N., and G. Asboe-Hansen. 1973. New method for quantitative determination of uronic acids. *Anal. Biochem.* **54**:484-489.
- Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**:315-323.
- Cadmus, M. C., H. J. Gasdorf, A. A. Lagoda, R. F. Anderson, and R. W. Jackson. 1963. New bacterial polysaccharide from *Arthrobacter*. *Appl. Microbiol.* **11**:488-492.
- Callet, F., M. Milas, and M. Rinaudo. 1987. Influence of acetyl and pyruvate contents on rheological properties of xanthan in dilute solution. *Int. J. Biol. Macromol.* **9**:291-293.
- Collins, E. A., J. Bares, F. W. Bares, and F. W. Billmeyer, Jr. (ed.). 1973. *Experiments in polymer science*, p. 397-398. John Wiley & Sons, Inc., New York.
- Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**:349-356.
- Gasdorf, H. J., R. G. Benedict, M. C. Cadmus, and R. F. Anderson. 1965. Polymer-producing species of *Arthrobacter*. *J. Bacteriol.* **90**:147-150.
- Ginsburg, V. 1964. Sugar nucleotides and the synthesis of carbohydrates. *Adv. Enzymol.* **26**:35-88.
- Hägström, L., and C. Förberg. 1986. Significance of an extracellular polymer for the energy metabolism in *Clostridium acetobutylicum*: a hypothesis. *Appl. Microbiol. Biotechnol.* **23**:234-239.
- Hassler, R. A., and D. H. Doherty. 1990. Genetic engineering of polysaccharide structure: production of variants of xanthan gum in *Xanthomonas campestris*. *Biotechnol. Prog.* **6**:182-187.
- Jeanes, A., C. A. Knutson, J. E. Pittsley, and P. R. Watson. 1965. Extracellular polysaccharide produced from glucose by *Arthrobacter viscosus* NRRL B-1973: chemical and physical characteristics. *J. Appl. Pol. Sci.* **9**:627-638.
- Knutson, C. A., and A. Jeanes. 1968. A new modification of the carbazole analysis: application to heteropolysaccharides. *Anal. Biochem.* **24**:470-481.
- Lawson, C. J., and I. W. Sutherland. 1978. Polysaccharides, p. 327-392. In A. H. Rose (ed.), *Economic microbiology*, vol. 2. Primary products of metabolism. Academic Press, London.
- Linton, J. D. 1990. The relationship between metabolite production and the growth efficiency of the producing organism. *FEMS Microbiol. Rev.* **75**:1-18.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
- McComb, E. A., and R. M. McCready. 1957. Determination of acetyl in pectin and in acetylated carbohydrate polymers. Hydroxamic acid reaction. *Anal. Chem.* **29**:819-821.
- Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31**:426-428.
- Okutani, K. 1984. Structural investigation of the capsular polysaccharide produced by *Cryptococcus* isolated from sea water. *Bull. Jpn. Soc. Sci. Fish.* **50**:1407-1412.
- Petersen, G. R., G. A. Nelson, C. A. Cathey, and G. G. Fuller. 1989. Rheologically interesting polysaccharides from yeasts. *Appl. Biochem. Biotechnol.* **20**:845-867.
- Robyt, J. F., and B. J. White. 1987. *Biochemical techniques. Theory and practice*. Wadworth, Inc., Belmont, Calif.
- Royer, J. C., and J. P. Nakas. 1987. Production of mycelial protein and hydrolytic enzymes from paper mill sludges by cellulotic fungi. *J. Ind. Microbiol.* **2**:9-13.
- Royer, J. C., and J. P. Nakas. 1989. Xylanase production by *Trichoderma longibrachiatum*. *Enzyme Microb. Technol.* **11**:405-409.
- Royer, J. C., and J. P. Nakas. 1991. Purification and characterization of two xylanases from *Trichoderma longibrachiatum*. *Eur. J. Biochem.* **202**:521-529.
- Sandford, P. A. 1979. Exocellular microbial polysaccharides. *Adv. Car. Chem. Biochem.* **36**:265-313.
- Shimizu, N., M. Tomoda, R. Gonda, M. Konari, N. Takanashi, and N. Takahashi. 1989. The major pectic arabinogalactan having activity on the reticuloendothelial system from the roots and rhizomes of *Saposhnikovia divaricata*. *Chem. Pharm. Bull.* **37**:1329-1332.
- Sloneker, J. H., D. G. Orentas, C. A. Knutson, P. R. Watson, and A. Jeanes. 1968. Structure of the extracellular bacterial polysaccharide from *Arthrobacter viscosus* NRRL B-1973. *Can. J. Chem.* **46**:3353-3360.
- Sutherland, I. W. 1977. *Surface carbohydrates of the prokaryotic cell*, p. 88. Academic Press, Inc., New York.
- Sutherland, I. W. 1987. Polysaccharide modification—a physiological approach, p. 71-79. In M. Yalpani (ed.), *Industrial polysaccharides: genetic engineering, structure/property relations and applications*. Elsevier Science Publishers B.V., Amsterdam.
- Tait, M. I., I. W. Sutherland, and A. J. Clarke-Sturman. 1986. Effect of growth conditions on the production, composition and

- viscosity of *Xanthomonas campestris* exopolysaccharide. *J. Gen. Microbiol.* **132**:1483–1492.
31. **Touchstone, J. C., and M. F. Dobbins.** 1983. Practice of thin layer chromatography, 2nd ed., p. 185. John Wiley & Sons, Inc., New York.
 32. **Wells, J.** 1977. Extracellular microbial polysaccharides—a critical overview, p. 299–313. *In* P. A. Sandford and A. Laskin (ed.), Extracellular microbial polysaccharides. American Chemical Society, Washington, D.C.
 33. **Yalpani, M., and P. A. Sandford.** 1987. Commercial polysaccharides: recent trends and developments, p. 311–335. *In* M. Yalpani (ed.), Industrial polysaccharides: genetic engineering, structure/property relations and applications. Elsevier Science Publishers B.V., Amsterdam.