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Hemicellulose Bioconversion to Polyanionic Heteropolysaccharides

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ABSTRACT

Anionic polysaccharides, traditionally obtained from plant or algal sources, have a variety of commercial uses. Such gums from microorganisms have received increased recent interest. We have initiated a program to investigate the bioconversion of pentosans to rheologically useful anionic extracellular polysaccharides (AEPS). A number of earlier-described species, including *Cryptococcus laurentii, Klebsiella pneumoniae, Arthrobacter viscosus,* and *Pseudomonas* ATCC 31260, appear to have potential in this regard. These organisms can individually convert either xylose, enzymatic oligomeric hemicellulose digests, dilute mineral acid hemicellulose ("TVA") hydrolysates, or a five-monosaccharide mixture simulating sulfite process liquors to AEPS. The formation parameters, compositions, mol-wt distributions, and the intrinsic viscosities of these purified AEPS are exemplified. Substitution of pentose as the major substrate for glucose can result in changes in mol-wt distribution or in the percentage of noncarbohydrate substituents in some AEPS. Pursuit of these observations may lead to interesting structure–property relationships and toward rheological applications for pentosan-derived AEPS.

Index Entries: Hemicellulose utilization; anionic extracellular polysaccharides; pentose bioconversions; viscoelastic properties.

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INTRODUCTION

The conversion of biomass to useful chemicals or fuels is a long-standing goal whose pursuit has been cyclical with an intensity that oscillates with wars, materials, and energy shortages, or with other political and economic factors. The intrinsic advantages of biomass over fossil mass and the spectrum of realizable products therefrom have been extensively reviewed over the past decade (cf *1–3*). Of the major biopolymers from renewable global photosynthate, e.g., lignin, cellulose, and hemicellulose, the latter is the most readily depolymerized into its components in which xylose, xylooligosaccharides, and mannose predominate. Yet there has been minimal technology transfer using xylan bioconversions (*4*), likely owing to the fact that xylose is not a universally metabolizable monosaccharide. Nevertheless, there have been numerous studies on the transformation of pentose-rich substrates into ethanol and other liquid fuels (*5*), to xylonic acid (*6*), or more traditionally to microbial cell mass.

Based on recent progress in process engineering (*7*), it can be anticipated, in addition to traditional paper mill effluents, that an increasing variety of pentose-enriched feedstocks will become available from hardwood sources. Thus, for example, the steam explosion pretreatment releases some of the acetyl groups in xylans, which in turn lowers the pH resulting in xylose and water-soluble xylose oligomers. Various dilute acid pretreatments of agricultural residues, hybrid poplar, and hardwood sawdust (*8–10*) can give rise to reducing sugar mixtures in which xylose comprises up to 80%. Solvent delignification pretreatment methodologies (organosolv pulping), which now appear to be economically feasible for small-volume pulping plants, also can provide relatively homogeneous hemicellulose fractions (*11,12*), which in turn are amenable to either dilute acid or enzymatic hydrolyses. The current major chemical transformations for hemicellulose center on furfural relay synthons.

In parallel with the foregoing advances, the development of microbial polysaccharide gums for various commercial applications is also proceeding rapidly (*13–15*). Such anionic polysaccharides have been shown to have food industry, clinical, pharmaceutical, oil well drilling, drag-reducing, and other rheological applications with growing markets, and can command specialty chemical prices. Since it is generally assumed that global photosynthesis produces, annually, some 5×10^{10} tons of hemicelluloses, it has been our objective to investigate the bioconversion of pentosans obtained by the above-delineated procedures into value-added heteropolysaccharides. To date, our preliminary studies on the bioconversion of hemicellulose to anionic extracellular polysaccharides (AEPS) of potential use have been carried out with the following microorganisms: *Cryptococcus laurentii* var. *flavescens* (*16*); *Klebsiella pneumoniae* ATCC 31646 (*17*); *Pseudomonas* sp. ATCC 31260 (*18*), and with *Arthrobacter viscosus* NRRL-B1973 (*19*). The exact repeating unit of the glucose-grown capsular polysaccharide of *C. laurentii* (NRRL-Y-1401) was delineated as follows:

$$\begin{array}{c} \beta\text{-D-Xyl}p \\ 1 \\ \downarrow \\ 6 \\ \text{—3)-}\alpha\text{-D-Man}p\text{-(1}\rightarrow\text{3)-}\alpha\text{-D-Man}p\text{-(1}\rightarrow\text{3)-}\alpha\text{-D-Man}p\text{-(1—} \\ 2 \\ \uparrow \\ 1 \\ \beta\text{-D-Glc}p\text{A} \quad + \text{OAc} \end{array} \qquad (1)$$

The probable structural repeating unit of this *Klebsiella* species EPS is (*20*):

$$\rightarrow\text{3)-}\alpha\text{-D-Galp-(1}\rightarrow\text{3)-}\alpha\text{-D-Galp A-(1}\rightarrow\text{3)-}\alpha\text{-L Fucp(1—} \qquad (2)$$

whereas the repeating unit of *Arthrobacter viscosus* EPS is:

$$O\text{-}\beta\text{-D-Manp A-(1}\rightarrow\text{4)-}\beta\text{-D-Glup-(1}\rightarrow\text{4)-D-Galp- 25\% }O\text{-acetyl} \qquad (3)$$

Pseudomonas #31260 AEPS is a complex succinoglycan whose structure remains to be completely elucidated. These particular cultures have now been shown to be capable of forming AEPS from several pentosan sources. In specific instances, these biopolymers exhibited modified molecular and solution properties, especially in comparison to their counterparts formed during growth on glucose. This communication delineates representative data concerning these facets.

MATERIALS AND METHODS

Organisms and Growth Conditions

The yeast and bacterial isolates employed in this investigation were maintained on potato-dextrose or nutrient broth agar slants. To ensure against strain deterioration for AEPS production levels, stock cultures were also lyophilized in skin milk. A modified liquid growth medium (*21*) was used for polysaccharide production and contained the following (g/L^{-1}): peptone, 1.0; yeast extract, 1.0; NH_4CL, 0.1; Na_2HPO_4, 0.6; KH_2PO_4, 0.4; and $MgSO_4 \cdot 7H_2O$, 0.2. Trace elements were added to a final concentration (μM) of: $FeSO_4 \cdot 7H_2O$, 0.036; H_3BO_3, 0.097; $CoCl_2 \cdot 6H_2O$, 0.017; $CuSO_45H_2O$, 0.08; $MnSO_4H_2O$, 0.019; and $ZnSO_4 \cdot 7H_2O$, 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. The filter-sterilized carbon sources were added aseptically to final concentrations of 1.0–3.0% (w/v). Liquid cultures were inoculated with 2–3 d starter cultures to final concentrations of 0.2–2.0% (v/v). These cultures were usually incubated at 28°C with shaking at 150 rpm. Fermenter runs (5.0 L) in a BioFlow II batch/continuous fermenter (New Brunswick Scientific, Edison, NJ) were conducted at 28°C, agitated at 200–600 rpm, aerated at a flow of 2–4 L/min, and seeded with a 2% 1-d-old inoculum. Microbial

growth was measured as CFUs by plating on complete medium or by absorbance measurement at 540 nm.

Polysaccharide Determination and Isolations

Production of AEPS was followed by a modified poly(hexamethylene-biguanidinium) chloride technique (*22*). For AEPS isolations, cultures were diluted 1:2 with distilled H_2O and centrifuged at 27,000 × *g* for 30 min. Hexadecyl trimethylammonium bromide was added to the supernatant to a final concentration of 0.175% (w/v). The gelatinous acidic extracellular polysaccharide precipitate was collected by centrifugation at 27,000 × *g* for 30 min. The clear supernatant was removed and discarded. The polysaccharide pellet was resuspended in 2*M* NaCl, precipitated with 3 vol of 95% ethanol, and dialyzed against deionized H_2O. To 1-mL aliquots of purified acidic polysaccharide solution (0.1–2.0 mg/mL) in 25-mL Erlenmeyer flasks were added 100 μL of 2.5% sodium tetraborate and 2 mL of 0.05% cetylpyridinum chloride in aqueous 0.05% sodium acetate. The flasks were then covered with parafilm and shaken at 120 rpm for 5 min. Next, a 1-mL portion was pipeted into a 1.5-mL plastic tube and centrifuged for 1 min to remove precipitated acidic polysaccharides. Five hundred microliters of the supernatant were then added to 2.5 mL distilled water, vortexed, and the absorbance was read at 259 nm in a Milton Roy Spectronic 1001 spectrophotometer. Extracellular anionic polysaccharide concentrations were extrapolated from standard calibration curves obtained from relevant AEPS or from xanthan gum.

Analytical Procedures

These generally followed the techniques for exopolysaccharide analyses reviewed in (*23*). Specifically, total carbohydrate was determined using the phenol-sulfuric method. To 1 mL of appropriately diluted sample, 0.5 mL of 5% phenol and 2.5 mL of concentrated sulfuric acid were added. Xylose utilization was monitored by a specific colorimetric method for pentoses (*24*) based on the formation of furfural from pentoses in acetic acid containing thiourea at 70°C. In this method, 1 mL of *p*-bromoaniline reagent was added to 2.0 mL of samples and standards. The controls were incubated in the dark at room temperature, whereas the test samples were incubated at 70°C for 10 min, rapidly cooled in an ice water bath, and then incubated in the dark for 70 min at room temperature. All the samples were read at 520 nm. Protein was determined by the Lowry method.

Uronic acid was determined by both the carbazole and the *m*-hydroxydiphenyl reactions (*25,26*). Acetyl determinations were carried out via the hydroxamic acid reaction (*27*). Pure glucose pentaacetate served as standard. A freshly prepared mixture (1:1) of 9.4% NaOH and 3.75% hydroxylamine (w/v) in distilled water was added (2 mL each) to volumetric flasks

(25 mL) with 5-mL solutions of standards or of samples. After 5 min, 5 mL of acid methanol solution were added, and these mixtures were brought up to the final volume by addition of ferric perchlorate solution. After 5 min, absorbance was read at 520 nm vs a water blank. Pyruvate and succinate residues were determined by HPLC methods (*28,29*).

Monomeric sugars from polysaccharides were prepared for analysis by hydrolyses with trifluoroacetic acid. Five milligrams of each purified polysaccharide were suspended in 0.5 mL of 2*M* trifluoroacetic acid and hydrolyzed for 4 h at 100°C using a heating block. Samples were then dried with a stream of nitrogen gas and reconstituted in 0.5 mL deionized H_2O. The component sugars were analyzed using TLC and HPLC techniques.

Chromatographic Techniques

Thin-Layer Chromatography

Each of the hydrolyzed polysaccharides, at a concentration of 10 mg/mL, was spotted (5 μL) on Whatman K5 silica gel glass plates. The solvent system consisted of *n*-butanol, pyridine, and water (6:4:3). Glass chromatography chambers were sealed with plastic wrap and a glass cover for the duration of the separation. The TLC plates were sprayed evenly with a reagent consisting of 3% (w/v) *p*-anisidine hydrochloride prepared in *n*-butanol, air dried, and heated at 100°C for 10 min.

High-Performance Liquid Chromatography

Hydrolyzed polysaccharide samples (10 mg/mL) were analyzed using a Perkin Elmer Series 10 Liquid chromatograph (Norwalk, CT). Filtered samples of 20 μL were loaded on an Aminex HPX-87P (Bio-Rad, Richmond, CA) monomer column (300×7.8 mm) with Carbo-P microguard cartridges equilibrated at 85°C, and run at 0.6 mL/min using a mobile phase of deionized H_2O. Polysaccharide components were detected at 192 nm with a Perkin-Elmer LC-75 spectrophotometric detector and by using a Perkin-Elmer LC-25 RI detector. Monosaccharide quantitations were obtained using a Hewlett-Packard Model 3394A peak integrator (Avondale, PA).

Gel Permeation Chromatography

Polysaccharide molecular weights were determined following methods previously described (*30*). A 1.5 cm×100 cm glass Econo-column from Bio-Rad was packed with Sephacryl S-400 HR obtained from Sigma. The samples (0.5 mL) containing 2 mg of polysaccharide were eluted by gravity with MOPS buffer. Fifty-drop fractions (1.3 mL) were collected using a Gilson model FC-80K fractionator (Middleton, WI). Fractions were analyzed for total carbohydrate by the phenol-sulfuric method. Dextran standards of approx 6000, 15,000–20,000, 40,000, and 70,000 mol wt were obtained from Fluka Chemical Corp. (Ronkonkoma, NY) and, like standards of approx 170,000, 600,000, and 2,000,000 mol wt were obtained from Polysciences, Inc. (Warrington, PA).

Viscosity Determinations

Polysaccharides were used at a concentration of 0.25% (w/v) in 0.15 *M* NaCl. Intrinsic viscosity was measured over four separate concentration dilutions at 25°C initially using a Cannon-Ubbelohde type viscometer size 75 from which intrinsic viscosity measurements were calculated. Alternatively, rheological measurements were made using a Brookfield (Stoughton, MA) viscometer model LDVT II at 25°C. Polysaccharide viscosities (cps) were compared over various polymer concentrations from 0.0 to 1.5% (w/v) in double-distilled water. Shear rates were varied from 0–18 (s^{-1}).

Carbon Sources

Reagent grade glucose, xylose, and xylan (from oat spelts) were obtained from Sigma Chemical Co. (St. Louis, MO). The xylan was enzymatically hydrolyzed to a mixture of xylooligosaccharides using a crude xylanase preparation (*31*) from *Trichoderma longibrachiatum*. Crude enzyme (250 μL) was added to a 5% (w/v) 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 4000 *g* for 15 min to remove insoluble xylan. The clear yellow xylan hydrolysate was filtered through a 0.22-μm pore-sized filter (Millipore Corp.) and lyophilized to dryness.

RESULTS

We have employed a limited screening process with several dozen organisms previously reported to produce viscous anionic extracellular polysaccharides, primarily from glucose, as well as via selective culturing techniques, to identify yeast and bacterial strains that can utilize xylose or xylose-rich substrates. The composition of the initial test medium is detailed in Materials and Methods. This communication is a descriptive overview of our findings, to date, with four such isolates, which are AEPS producers on representative pentosan sources.

Cryptococcus laurentii var. *flavescens* produces ca. 7 g/L of crude AEPS from xylose-rich media after 96 h of growth. When grown on various pentosan substrates, *C. laurentii* produced more polysaccharide per viable cell on simulated sulfite waste sugars than on any other pentosan substrate tested. Analysis of the purified AEPS formed on various carbon sources revealed a constant monomeric composition consisting of xylose, mannose, glucuronic acid, and acetate. However, this organism did not appear to biosynthesize significant amounts of AEPS on neutralized mineral acid hydrolysates of hemicellulose ("TVA" hydrolysates [*32*]). During growth, the chronologic appearance of AEPS from *C. laurentii* is shown in Fig. 1. The organism appears to form this extracellular biopolymer in a "diauxic" fashion, which is consistent with the composition of the growth melange,

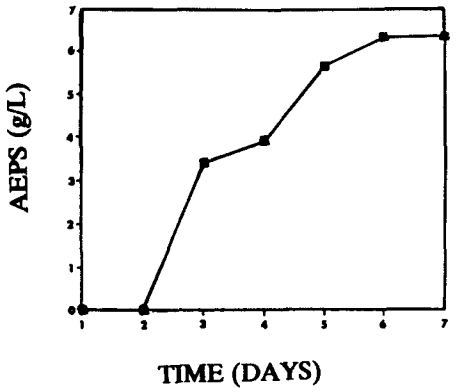

Fig. 1. Extracellular polysaccharide production by *C. laurentii* when grown on simulated sulfite waste liquor carbohydrate mixture.

i.e., mannose/xylose/galactose/glucose/arabinose; 12:6:5.5:4:2.5, specified by Wayman and Yu (*33*). Whether this strain can actually utilize spent sulfite liquor in a comparable fashion remains to be seen, especially in light of an earlier report (*34*) that steam stripping of this material was necessary for pullulan production by *Aureobasidium pullulans*.

Among a number of designated *Klebsiella pneumoniae* strains, some of which formed no AEPS when grown on xylose, isolate ATCC 31646 was the best AEPS producer. The most likely structure of this AEPS is a repeating trisaccharide wherein galacturonic acid, fucose, and galactose are linked by α; 1→3 bonds, with acetyl substitutions (*20*). This isolate synthesizes AEPS from enzymatically hydrolysed hybrid poplar or oat spelt xylans, and from glucose, all of constant monomeric composition regardless of substrate used. Optimal production of AEPS on xylose was determined under the following conditions: minimal medium buffered with 100 m*M* sodium phosphate to a pH of 7.3, 1% xylose at a C:N ratio of approx 10:1, growth in a vigorously aerated fermenter at 28°C for 4–5 d. In batch fermentation experiments, conversion efficiencies exceeded 60%, and AEPS concentrations in excess of 7.0 g/L crude product were achieved. Figure 2 shows data from a typical fermenter experiment. Gel permeation chromatographic analyses of AEPS obtained from glucose, xylose, or an enzymic hydrolysate from poplar hemicellulose showed identical elution patterns.

Arthrobacter viscosus NRRL B-1973 produces viscous AEPS when grown on glucose (*19*) or in media containing xylose or xylan hydrolysates. Polysaccharide production on xylose was optimized to 10–12 g/L of crude product when the initial culture pH was 8.0 and total nitrogen was limited to 0.03%. The homogeneity of purified AEPS preparations was determined using PAGE tube gels (10% acrylamide) with visualization achieved using toluidine blue. AEPS from growth on each substrate were analyzed for monosaccharide components, percent acetyl, and uronic acid. Average

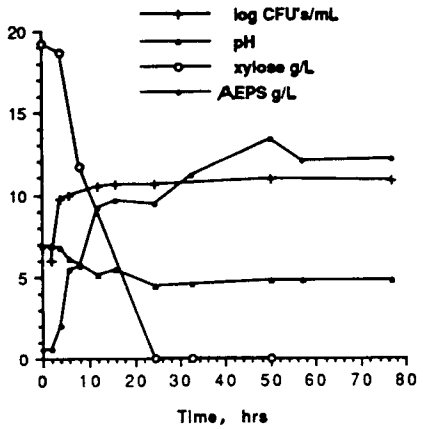

Fig. 2. Time course of growth, crude AEPS formation, and of substrate disappearance for *K. pneumoniae* cultured on 2% xylose medium in a 5-L fermentor. The pH was controlled continuously by addition of 5*M* NaOH.

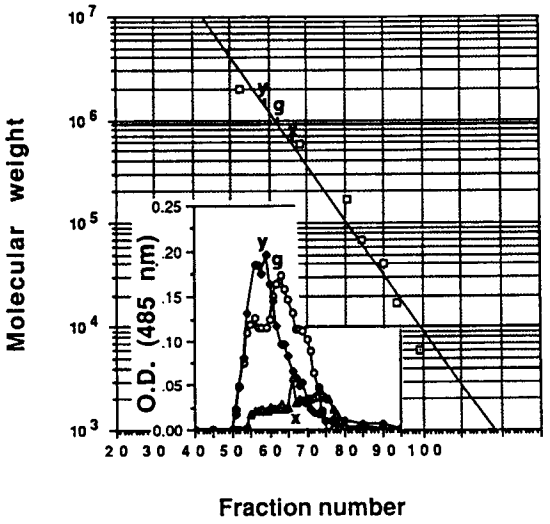

Fig. 3. Mol-wt distributions for *A. viscosus* AEPS from glucose (g), xylose (x), and from oligoxylosaccharides (y). The straight line relationship was obtained with known mol-wt standards (*see* Materials and Methods).

molecular weights were determined by gel exclusion chromatography (Fig. 3). Differences were apparent in degrees of acetylation, average mol wt, and intrinsic viscosities of the heteropolysaccharides produced from different carbon sources, whereas similarities were seen in charge, ultrastructure, and their constituent sugars (Table 1). Here, some intimation of a change in degree of noncarbohydrate substitution (i.e., percent acetyl groups) relative to the nature of the substrate utilized can be drawn. Whether there is a causal relationship between this observation and the parallel mol wt distributions given in Table 1 remains to be investigated.

Table 1
Molecular Characteristics of *A. viscosus* AEPS

Composition	Carbon source		
	Glucose	Xylose	Xylan hydrolysate
% Total carbohydrate	56.0±2.0	53.0±1.5	53.3±1.3
% Mannuronic acid	18.0±0.7	17.3±1.3	17.9±1.3
% Acetyl	24.4±0.8	29.3±1.9	20.0±0.5
Avg. mol. wt.	900 kDa	1500 kDa	600 kDa
$[\eta]^a$	19.25	25.27	8.63

[a]Intrinsic viscosity.

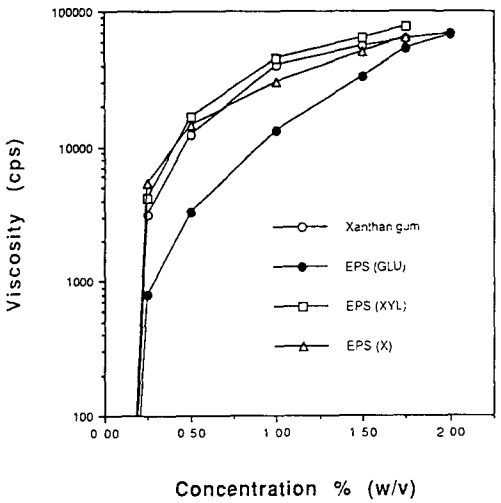

Fig. 4. Viscosity vs purified AEPS concentrations for a commercial xanthan gum preparation and for *A. viscosus* heteroglycans formed from glucose (GLU), xylose (X), and from oligoxylosaccharides (XYL). Shear rate, constant (0.44 rad s^{-1}).

A comparison of the gross rheological properties of xanthan gum and AEPS produced by *A. viscosus* from either glucose, xylose, or an enzymic digest of oat spelt hemicellulose is provided graphically in Fig. 4. It can be seen that the xylose-derived AEPS are approximately equivalent to a commercial xanthan preparation under these test protocols.

Pseudomonas ATCC #31260 synthesized extracellular acidic polysaccharides in shaken batch culture from four substrates: commercial xylose, the carbohydrate mixture simulating sulfite pulping waste (*20*), the enzymatic digest of xylan (*31*), and two first-stage dilute acid wood hydrolysates. Parameters optimized for AEPS formation by this isolate included: an initial pH of 8.4 with maintenance of pH over 6.5 for the 10-d duration of fermentation, a carbon to nitrogen ratio > 13/1, a phosphate concentration of 5.0 mg/mL, and an initial pentose level at 2.0%. Production

Table 2
Percent Composition of Purified AEPS
Produced by *Pseudomonas* #31260 from Various Substrates

Substrate	Carbohydrate	Protein	DNA	Acetyl	Pyruvyl	Succinyl	Total
Xylose	69.1	1.6	0.05	3.8	6.4	10.7	92
Simulated sulfite waste	81.7	1.5	0	3.1	5.5	11.4	103
Hemicellulose enzymic hydrolysate	60.1	6.7	0	2.8	5.8	18.6	94
Glucose[a]	72.3	n.a.[b]	n.a.[b]	3–4	5–9	n.a.[b]	n.a.[b]

[a]As characterized by Williams (34).
[b]Not available.

of AEPS under these conditions was biphasic, indicative either of sequential formation of exopolysaccharides of different structures or of an inductive lag for preferential monosaccharide utilization. For the AEPS collected at the end of growth on four different substrates, the percent compositional analysis are given in Table 2. Of particular interest was our finding, among the noncarbohydrate residues in addition to acetyl and pyruvyl residues (*35*), of the hitherto unreported dicarboxylic acid, succinate. By analogy, it should be pointed out that the exopolysaccharide of *Ps. marginalis* is also a succinoglycin (*36*). It would thus appear that the degree of substitution of the latter three residues in our succinoglycan is directly related to the nature of the monosaccharide substrate. Furthermore, the molecular weights of these individual AEPS, as determined by gel permeation chromatography, ranged from 1800 kDa for simulated sulfite waste, 2000 kDa for xylose, and to 4500 kDa for the enzyme-digested xylan.

Pseudomonas #31260 also grows well and produces AEPS on amended "TVA" first-stage mineral acid hydrolysates. The average composition of this substrate has been reported to contain xylose/glucose/galactose/arabinose/mannose/acetic acid/furfural/hydroxymethylfurfural in ratios of 43:9:3.3:2.9:3.4:11:0.3:0.9 g/L (*32*). In our hands, best results have been obtained when this material was diluted with phosphate buffer, pH 7.6, resulting in a total carbohydrate concentration of 1.0%, and yeast extract and peptone were added to produce final concentrations of 0.05% each. Using 24-h slant inocula, the data presented in Table 3 were obtained. Two tentative conclusions can be drawn: first, that the yeast extract-peptone amendment is not (in keeping with their relatively low concentrations) the substrate for AEPS formation, but more likely acts as a cofactor stimulus; and second, that inoculum size may ameliorate the generally known toxic activities of the aliphatic acids, furfural derivatives, and lignin components in such hemicellulose chemically catalyzed hydrolysates (*6,37*).

Table 3
Yield of Crude AEPS Formed by *Pseudomonas* #31260 on Stage I "TVA" Acid Hydrolysate (*36*)[a]

Inoculum size, mL	Total % carbohydrate	AEPS, g/L
0.20	1.0	11.2
0.40	1.0	11.8
1.0	1.0	12.3
2.0	1.0	16.6
1.0	0	n.d.[b]
2.0	0	n.d.[b]
0.20	1.0[c]	1.4
1.0	1.0[c]	2.9

[a]"TVA" hydrolysates adjusted to 1% total carbohydrate with phosphate buffer, pH 7.6, and amended with yeast extract and peptone, each to a final concentration of 0.05%. Flasks inoculated with 24-h slant cultures and incubated for 7 d. Crude AEPS precipitated with cetyltrimethylammonium bromide (approx 50% polysaccharide).
[b]None detected.
[c]Control flasks lacked yeast extr. and peptone.

DISCUSSION

Current advances in the biotechnology of hybrid biomass tree and crop production will yield lignocellulosic producers with rapid growth and other favorable qualities with respect to environmental tolerance. Concurrently, it is anticipated that parallel advances in process engineering will provide additional avenues to partially purified hardwood hemicelluloses and to their hydrolysis products. The research exemplified in this article is predicated upon an approach for the reintegration of essentially low-value xylans and xylose-containing process streams into value-added, biodegradable anionic polysaccharides. We have now established that a number of yeast and bacterial isolates can convert such starting materials into extracellular, rheologically useful, water-soluble polymers in yields approaching commercial developments. The advantages of this approach include the production of such polysaccharides under controlled fermentation conditions and the possible manipulation of product properties by genetic means.

The initial physiological phenomena required for xylose utilization by microorganisms involve specific transport of this monosaccharide (*38*), enzymatic redox steps, or an isomerization to provide D-xylulose, entrance into the pentose phosphate cycle via phosphorylation, and attendant cofactor regenerations. The subsequent biosynthesis of microbial extracellu-

lar polysaccharides that consist of repeating structural units usually involves a sequence of activation of primary residues via glycosyl nucleotide formation, the formation of glycosyl polyprenyl diphosphates, condensations of the latter to the repeating basic unit, modifications with functional noncarbohydrate substituents, assembly of the polymeric chains by elongation steps, and finally, extrusion and transport (*39,40*). It is obvious that genetic or metabolic feedback regulation of AEPS formation may take place at any of the foregoing intermediary stages and depends on total ATP demands (*39*). A particularly relevant example of such expression comes from investigations on the biosynthesis of the capsular polysaccharide of *Cryptococcus neoformans*, which is taxonomically related to the *C. laurentii* isolate, which we have shown to produce its AEPS from pentosan sources (Fig. 1). It was found with the former yeast (*41*) that the formation of the intermediary precursor UDP-glucuronate is strongly inhibited by UDP-xylose. Whether such kinds of metabolic regulatory interplay take place in the microorganisms studied here that form uronic acid-containing AEPS when xylose-grown is subject to further investigation.

For *A. viscosus* and *Ps.* strain #31260, substitution of pentose for hexose as the primary carbon source resulted in significant changes in the compositional content of the noncarbohydrate, acidic substituents in the resultant AEPS that were produced, as well as in differences of the degree of polymerization of the repeating heteroglycan subunits (Tables 1 and 2). Such variations in the ultimate composition of extracellular polysaccharide in relation to the nature of the carbon source have been noted for several gram-negative bacteria (cf. *42*). However, the biochemical aspects of the control of enzymatic biopolymer modifications remain, in general, to be elucidated. In the case of xanthan gum, which already has wide commercial application as a viscosifier, mutant strains have been genetically engineered (*43*) that produce such structurally variant AEPS. More specifically, it has been shown in this article that the degree of pyruvylation and acetylation affects the viscosimetric properties of these biopolymers. Thus, for our xylose-grown strains that make AEPS of significant structural differences in comparison to that from their hexose-grown fermentations, a more detailed study of correlations between the degree of basic heteroglycan backbone modifications and viscoelastic behavior would appear to be warranted. Such types of relationships have also been initiated for the *Rhizobium* heteropolysaccharides (*44*).

Further by analogy with recent biochemical genetic studies on the formation of xanthan gums, we look forward toward obtaining mutants of the microorganisms reported in this article that can overexpress AEPS formation, that will biosynthesize a spectrum of products with different degrees of polymerization, and that can exhibit even greater tolerance for the toxic byproducts (Table 3) found in chemical lignocellulose hydrolysates. Finally, using both yeast and bacterial isolates, the construction of recombinants that may also express xylanase genes can be explored. If

successful, the latter stratagem would effect an even more direct bioconversion of the renewable forest resource hemicellulose to useful heteroglycans.

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