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EXTRACELLULAR POLYSACCHARIDES OF *AZOTOBACTER VINELANDII*[1]

GARY H. COHEN[2] AND DONALD B. JOHNSTONE

Department of Agricultural Biochemistry, University of Vermont, Burlington, Vermont

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ABSTRACT

COHEN, GARY H. (University of Vermont, Burlington), AND DONALD B. JOHNSTONE. Extracellular polysaccharides of *Azotobacter vinelandii*. J. Bacteriol. **88**:329-338. 1964.—Extracellular polysaccharides synthetized by *Azotobacter vinelandii* strains 155, 102, and 3A were shown to be carboxylic acid heteropolysaccharides of apparent high molecular weight. Cells were grown in a nitrogen-free, mineral broth medium with 2% sucrose. Extracellular slime was recovered by centrifugation and purified by repeated alcohol precipitation and Sevag deproteinization. Capsular polysaccharide was recovered from washed cells by mild alkaline digestion. Methods of isolation and purification appeared to provide polysaccharide showing no evidence of heterogeneity when examined by chemical and physical methods. Infrared analysis of purified slime from the three strains suggested fundamental structural similarities. Colorimetric, paper chromatographic, and enzymatic analyses on both intact and acid-hydrolyzed slime polysaccharide indicated that the polymers contained in common galacturonic acid, [α] D-glucose, and rhamnose at a ratio of approximately 43:2:1, as well as a hexuronic acid lactone, probably mannurono-lactone. However, as shown by chemical and infrared analysis, minor differences did exist; namely, slime from strain 155 and 102 contained *o*-acetyl groups, whereas slime from strain 3A contained none. A sialic acid-like component (1.5% of dry weight of the polysaccharide, calculated as *N*-acetyl neuraminic acid), was found only in the slime of strain 155. Capsular polysaccharide composition closely resembled that for slime. It is of interest that the major slime components were identical whether the energy source provided for the cells was sucrose, glucose, fructose, or ethanol.

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[2] Present address: School of Veterinary Medicine, University of Pennsylvania, Philadelphia.

Azotobacter vinelandii, under most cultural conditions, synthesizes copious amounts of extracellular polysaccharide. This polysaccharide occurs morphologically in two distinct forms: (i) discrete capsules adhering to the cell walls, and (ii) as amorphous slime found loosely attached to the capsules and in the culture medium free from the cells.

Within recent years, reports have appeared implicating *A. vinelandii* polysaccharide with various phenomena associated with this species. Wyss, Neuman, and Socolofsky (1961) reported that *A. vinelandii* cysts, which conceivably were formed from extracellular polysaccharide synthesized by this species, consisted primarily of rhamnose and glucose components. Eklund and Wyss (1962) demonstrated the presence of a capsule-digesting enzyme formed when *A. vinelandii* cells were infected with bacteriophage. Preliminary tests by these authors on the chemical composition of the capsules indicated the presence of glucuronic acid and an unidentified component. Recently, Cohen and Johnstone (1963*a*) presented evidence that the acid reaction produced in culture medium by *A. vinelandii* was associated with extracellular slime synthesis. They reported galacturonic acid as the major component of the slime.

Earlier reports on the chemical composition of *A. vinelandii* polysaccharide indicated that the capsules consisted of glucose and rhamnose (Kaufman and Rapaske, 1958), whereas the cell-free slime, as stated by Kaufman (1960), consisted of glucose, a trace of uronic acid, and no methyl pentose.

Knowledge pertaining to the chemical composition of the extracellular polysaccharide of *A. vinelandii* is basic to an understanding of the polymer's functional role in this species. Furthermore, such information may provide additional distinguishing characteristics for the differentiation of the often confused species, *A. vinelandii* and *A. agilis* (Johnstone, 1962).

Results presented here concern some studies of the physical and chemical properties of extracellular polysaccharide produced by several strains of *A. vinelandii*. A preliminary report of part of this work was presented (Cohen and Johnstone, 1963*b*).

MATERIALS AND METHODS

Growth of the organisms. The organisms used throughout this study were *A. vinelandii* strains 3A (ATCC 12837), 102 (ATCC 9046), and 155 (ATCC 12518). Burk's nitrogen-free broth (Wilson and Knight, 1952) at pH 7.0 supplemented with 2% sucrose was inoculated with the appropriate strain growing in the logarithmic phase. Cultures were incubated at 31 C in 7.5-liter New Brunswick fermentors with sterile moist air supplied at the rate of 4 liters per min, and an impellor rotation of 130 rev/min. Incubation was discontinued and the polysaccharide was harvested when the reaction of the culture medium reached pH 5.5 (Cohen and Johnstone, 1963*a*). This usually occurred in about 5 days.

Isolation and purification of extracellular polysaccharide. The viscous culture medium was diluted onefold with distilled water, and the cells were removed in a Servall continuous-flow centrifuge at 18,400 × *g*. The slime polysaccharide, designated hereafter as SP, was precipitated from the culture supernatant fluid by the addition of 4 volumes of cold (−10 C) acidified (HCl) absolute ethanol. The precipitated SP was removed, washed with absolute ethanol, air-dried, and redissolved in water. Dissolution of SP was facilitated by mixing in water for 12 hr at 4 C. This was then centrifuged at 18,400 × *g* for 1 hr at 4 C to remove undissolved material. The above purification procedures were repeated three times. SP recovered from *A. vinelandii* 155 and 102 was purified by these methods. To obtain a highly purified preparation for studying chemical and physical properties in greater detail, SP from strain 3A was subjected to the following additional procedures. Protein was removed by Sevag (Sevag, Lackman, and Smolens, 1938) extraction. Sevag deproteinization was discontinued when protein was no longer detected by the method of Lowry et al. (1951). The turbid, opalescent nature of aqueous solutions of SP was completely removed by this treatment, leaving a water-clear solution. At this point, 1% (w/v) NaCl was added to insure complete precipitation, and SP was precipitated with cold acidified ethanol. The precipitate was redissolved, centrifuged at 18,400 × *g* for 3 hr at 4 C to remove undissolved material, and reprecipitated. Precipitated SP from each strain was washed successively in 80% ethanol, absolute ethanol, acetone, and ether, and was dried to constant weight in vacuo at room temperature.

Capsular polysaccharide was removed from washed cells by adjusting the reaction of the medium to pH 9.0 with NaOH and incubating the cells on a rotary shaker for 1 hr at room temperature. The alkaline digestion mixture was centrifuged, the cell mass was discarded, and the polysaccharide was isolated and purified by the above methods. For studies of SP synthesized from ethanol, strain 3A was grown on 1% (v/v) ethanol as the sole carbon and energy source with an additional 1% added after 16 hr of incubation as described by Kaufman (1960).

Methods for determining polysaccharide homogeneity. The crude supernatant fluid containing SP was titrated with a 3% (w/v) solution of ethylhexadecyldimethyl ammonium bromide until precipitation of acidic polysaccharide no longer occurred. The culture medium was cleared of precipitated SP by centrifugation and refrigerated overnight, and the titration procedure was repeated. After all traces of precipitate were removed, 4 volumes of cold (−10 C) ethanol were added to precipitate any remaining polysaccharide. Detergent was removed from SP by washing with methanol.

SP was analyzed electrophoretically with 5% acrylamide gel as a supporting medium, according to the methods of Davis (1963). The polysaccharide was located on acrylamide gel with 0.06% (w/v) Toluidine Blue in 7% (v/v) acetic acid. Destaining was accomplished with 7% (v/v) acetic acid.

Ultracentrifugal studies were carried out in a 2.5 to 5.0% (v/v) linear glycerol density gradient at 39,000 rev/min with a SW 39 swinging-bucket rotor of a Spinco L preparative ultracentrifuge. The orcinol test (Mejbaum, 1939) was used to estimate polysaccharide concentration in the presence of glycerol.

Physical methods. Infrared analyses of SP incorporated into KBr pellets were done in a Perkin-Elmer model 21 double-beam spectrophotometer. For these analyses, SP was precipitated from neutral solution and air-dried.

All other spectrophotometric measurements were performed in a Beckman model DU spectrophotometer with cuvettes having a light path of 1.0 cm. Molecular weight estimation of SP, dissolved in 0.5 M NaCl, was performed, by use of Sephadex gel filtration, according to Flodin (1962).

Chemical methods. Phosphorus and nitrogen determinations were carried out by the Fiske and SubbaRow (1925) and micro-Kjeldahl techniques, respectively. Protein concentration was estimated as described by Lowry et al. (1951) with purified *A. vinelandii* 3A protein as a standard. The protein standard was prepared according to Racusen and Johnstone (1961), except that the method of Lowry et al. (1951) was employed instead of the biuret reaction. Hexuronic acid was detected and identified by the following methods: carbazole method, modified carbazole test for galacturonic acid, the reaction of glucuronic acid with thioglycolic acid and sulfuric acid in the presence of mannose, and the reaction of galacturonic acid with L-cysteine and sulfuric acid according to the methods of Dische (1962). Quantitative determinations of hexose and methylpentose were accomplished by two different procedures. (i) Determinations were carried out on the intact SP by use of the primary L-cysteine-sulfuric acid reaction for hexose (Dische, Shettles, and Osnos, 1949) and the 10-min modification of the reaction of methylpentose with L-cysteine and sulfuric acid (Dische and Shettles, 1948). Adequate internal controls were included to account for both nonspecific color reactions caused by sulfuric acid treatment of the polysaccharide and the carbohydrate content of the polymer. (ii) The sugar moieties, obtained from acid hydrolysis of SP, were separated by paper chromatography, extracted from the paper, as recommended by Smith and Montgomery (1959), and assayed by the phenol-sulfuric acid method of Dubois et al. (1951).

O-acetyl groups were estimated according to Hestrin (1949). The thiobarbituric acid assay method of Warren (1959) was employed for the determination of sialic acid. A purified sample of *N*-acetyl neuraminic acid isolated from bovine submaxillary gland was the gift of R. C. Woodworth, University of Vermont. Total carbohydrate was estimated by the phenol-sulfuric acid method of Dubois et al. (1951), and reducing sugar was determined by the colorimetric procedure of Nelson (1944) and Somogyi (1952). Glucose was used as a standard for both methods.

Chromatography. Paper chromatography was carried out by descending and ascending techniques on Whatman no. 1 and 3 MM paper. The following solvent systems were used: (A) *n*-butanol–pyridine–water (9:5:8, v/v); (B) ethyl acetate-pyridine-water (12:5:4, v/v); (C) isopropanol-butanol-water (14:2:4, v/v); (D) ethyl acetate-pyridine-acetic acid-water (5:5:1:3, v/v). The sugars and sugar derivatives were located and identified with aniline hydrogen phthalate (Partridge, 1949), aniline diphenyl amine (Smith, 1958), hydroxamic acid spray for lactones and esters (Abdel-Akher and Smith, 1951), glucose oxidase reagent (Worthington Biochemical Corp., Freehold, N.J.; Salton, 1960), and 0.2% (w/v) ninhydrin in acetone for amino sugars.

Acid hydrolysis. Acid hydrolyses were carried out in Teflon-lined screw-capped tubes. Hydrolysates to be used for paper chromatography were prepared as follows. Polysaccharide was hydrolyzed in 2 N H_2SO_4 at 100 C for 5 hr. (A time course was run to obtain maximal reducing values.) The hydrolysate was neutralized with solid $Ba(OH)_2$, the precipitated $BaSO_4$ was removed by centrifugation, and excess Ba^{++} was removed by passing the supernatant fluid through a column containing Dowex 50 (hydrogen form).

For large-scale separation of SP components SP was hydrolyzed in 2 N HCl at 100 C for 5 hr, and the hydroylsate was filtered. The hydrolysate was evaporated to dryness under reduced pressure and redissolved; the procedure was repeated four times to remove HCl.

The acidic sugars were adsorbed on Dowex 1–2X (acetate form); the neutral sugars (neutral fraction) were eluted with distilled water, chromatographed on Whatman 3 MM with solvent B, and isolated according to Smith and Montgomery (1959). The acidic sugars (acidic fraction), after elution from the resin with 4 N acetic acid, were isolated by the same chromatographic techniques.

RESULTS

Homogeneity of polysaccharide. The addition of alcohol to the culture supernatant fluid of strain 3A, previously depleted of SP by detergent treatment, resulted in the formation of a white flocculant precipitate. This second precipitate (43

mg per liter of supernatant culture medium) was also demonstrated after alcohol precipitation of SP, but in both cases occurred only after 12 to 24 hr at 4 C. Analysis of the dialyzed second precipitate indicated a carbohydrate to protein ratio of 2.2, no uronic acid, and the presence of glucose. The precipitate was associated with the green fluorescent pigment of *A. vinelandii* and may be similar to the fluorescent peptide described by Bulen and Le Comte (1962). The yield of crude SP recovered after treatment with alcohol or detergent was 740 mg per liter of supernatant culture medium. These figures are relative to the culture batch, and represent an average of polysaccharide from several isolations. Recovery of SP immediately after alcohol precipitation appeared to separate the two precipitates, since the second formed only after a period of time in the cold.

The homogeneity of highly purified SP was examined by acrylamide gel "disc" electrophoresis. After electrophoresis, the gels were stained with Toluidine Blue. A slow-moving anionic band exhibiting metachromasy, which is indicative of acid polysaccharides, was observed along with a faintly stained area preceding the heavy band. The faster-moving area probably represented species of the acidic polymer of lower molecular weight, since it also exhibited metachromatic properties.

Sedimentation profiles of highly purified SP in a linear glycerol density gradient revealed a single symmetrical peak.

From these data, it would appear that the SP under investigation was homogeneous in composition.

Physical analysis. The infrared absorption spectra of SP in the dissociated form from strains 3A, 155, and 102 are illustrated in Fig. 1. The following functional group assignments were maed: OH, 3.0 μ; C—H, 3.45 μ; —CH_2CO—

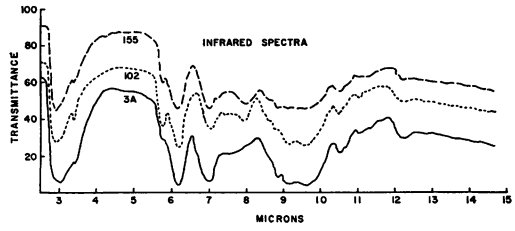

FIG. 1. *Infrared absorption spectra for extracellular polysaccharides produced by Azotobacter vinelandii strains 155, 102, and 3A.*

O—R, 5.77 and 8.1 μ; and O=C—$\bar{O}$, 6.25 and 7.1 μ (Levine, Stevenson, and Kabler, 1953). These spectra were quite similar, especially in the region 8.5 to 14.0 μ, which indicated a fundamental structural similarity. However, the spectra for SP 3A differed by the absence of ester linkage absorption at 8.1 μ, and only a slight shoulder at 5.77 μ which corresponded to the carbonyl band. Subsequent chemical tests (Hestrin, 1949) failed to detect *o*-acetyl groups in SP 3A, whereas positive results, as described below, were obtained for SP 155 and 102.

The ultraviolet spectrum of a 0.1% (w/v) aqueous solution of SP 3A revealed no maxima in the region 230 to 300 mμ. Molecular weight studies with various types of Sephadex (G-25 to G-200) suggested that the largest molecular weight of a species of the polymer was greater than 200,000, since it is excluded from a column of Sephadex G-200. This should be interpreted with caution, since one must assume that SP follows the principle of exclusion from Sephadex gel pores by size alone.

The highly viscous polysaccharide precipitated in the presence of multivalent cations, or when the pH reaction was adjusted to pH 3.5. These attributes, as pointed out by Whistler (1959), are indicative of carboxylic acid polysaccharides. Exhaustive dialysis of SP against water resulted in the formation of a firm gel.

Chemical analysis. SP possessed no reducing properties (Nelson, 1944; Somogyi, 1952) and a negligible amount of phosphorus (Fiske and SubbaRow, 1925). Paper chromatography of acid hydrolysates revealed four major components which were identified by the following methods as galacturonic acid, a lactone, rhamnose, and [α]D-glucose (Table 1).

The results of the carbazole test (Dische, 1962) on SP indicated a high concentration of uronic acid. Furthermore, the modified carbazole method (Dische, 1962) demonstrated that galacturonic acid was present. Figure 2 illustrates the absorption spectra for unhydrolyzed SP, an acidic fraction isolated from an acid hydrolysate, and authentic samples of glucuronic and galacturonic acid.

Further presumptive evidence for galacturonic acid was indicated by a positive L-cysteine-sulfuric acid test (Dische, 1962). However, since a uronic acid lactone was present (as described below), and presumably galacturonic acid does

TABLE 1. *Paper chromatography of sugar residues from a hydrolysate of extracellular polysaccharide**

Sugar	Solvent system			
	n-Butanol–pyridine–water	Ethyl acetate–pyridine–water	Isopropanol–butanol–water	Ethyl acetate–pyridine–acetic acid–water
Glucose	100	100	100	100
Hexose spot	100	100	99	101
Rhamnose	148	183	171	139
Methyl pentose spot	148	184	170	140
Glucuronolactone	156	242	158	180
Mannuronolactone	126	213	145	152
Lactone spot	127	212	145	153
Galacturonic acid	29	32	17	36
Uronic acid spot†	27	33	17	Tailing

* Results are expressed as $R_{glucose}$ values.

† R_G values for glucuronic acid were similar to those for the uronic acid spot.

not form a lactone, whereas glucuronic and mannuronic acids do (Percival, 1963), the presence of more than one uronic acid was investigated. Results of the thioglycolic-sulfuric acid test in the presence of mannose (Dische, 1962) indicated that glucuronic acid was not part of the polymer. Figure 3 illustrates the absorption spectra of the acidic fraction and authentic glucuronic and galacturonic acid. Unhydrolyzed SP also gave a spectral curve similar to that of galacturonic acid.

The hydroxamic acid spray (Abdel-Akher and Smith, 1951) was utilized to identify a component found in acid hydrolysates as a lactone. Infrared analysis of the isolated lactone indicated characteristic absorption peaks for the carboxylate ion (6.25 and 7.1 μ). The lactone was present on paper chromatograms irrigated in all of the

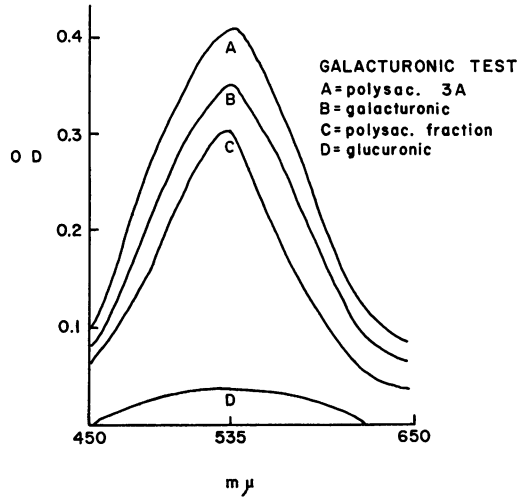

FIG. 2. *Absorption spectra for intact extracellular polysaccharide of strain 3A, galacturonic acid, an acidic fraction isolated from the polysaccharide hydrolysate, and glucuronic acid, in the modified carbazole test for galacturonic acid.*

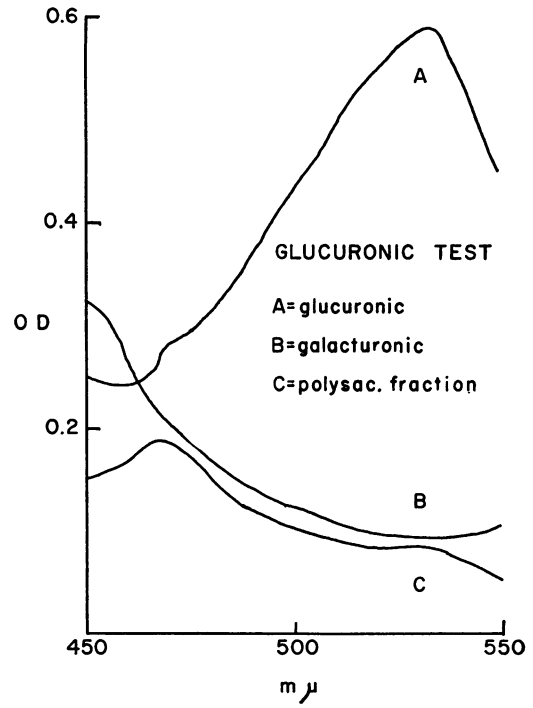

FIG. 3. *Absorption spectra for glucuronic acid, galacturonic acid, and an acidic fraction isolated from an extracellular polysaccharide hydrolysate of strain 3A, in the thioglycolic acid test for glucuronic acid.*

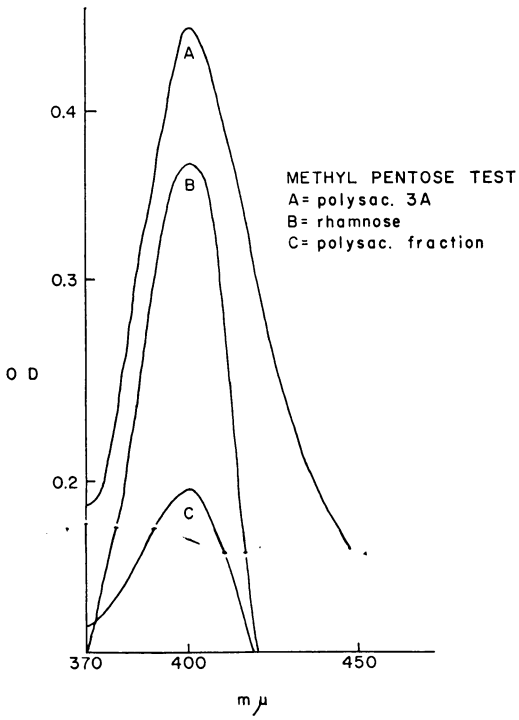

FIG. 4. *Absorption spectra for intact extracellular polysaccharide of strain 3A, rhamnose, and a methyl pentose isolated from the neutral fraction of the polysaccharide hydrolysate, in the cysteine-sulfuric acid test for methyl pentose.*

solvent systems. When the lactone was eluted from paper and rechromatographed in solvents A, B, C, or D, it dissociated into two components, one of which moved at the same rate as the lactone and a second slower-migrating component. Eluates of this second spot gave a positive carbazole test (Dische, 1962) for hexuronic acid and a negative modified carbazole test (Dische, 1962) for galacturonic acid. In addition, the lactone migrated slower than glucuronolactone in all solvents used and appeared to resemble closely the R_F values of mannuronolactone (Table 1).

Methyl pentose was indicated on the basis of chromatographic evidence (Table 1) and the L-cysteine-sulfuric acid test of Dische and Shettles (1948). Figure 4 illustrates identical absorption spectra for unhydrolyzed SP, a methyl pentose isolated from the neutral fraction of an acid hydrolysate, and an authentic sample of L-rhamnose. Rates of movement in four solvent systems, by ascending, descending, and co-chromatographic techniques, as well as identical color reactions with developing sprays, showed this component to be identical with rhamnose.

Hexose was indicated on the basis of chromatographic evidence (Table 1) and the primary cysteine-sulfuric acid method of Dische et al. (1949). Figure 5 illustrates identical absorption spectra for a hexose isolated from the neutral fraction of an acid hydrolysate and an authentic sample of D-glucose. The isolated hexose fraction was identified by paper chromatography with authentic D-glucose (Table 1). Since glucose and galactose have similar R_F values in most solvents, cochromatography, multiple ascending (solvent A), and descending (solvent B) paper chromatographic techniques were utilized to separate these two sugars. Furthermore, the isolated fraction gave a positive glucose oxidase test (Salton, 1960), which strongly indicated [α]D-glucose.

Thus far, the data presented have been for SP 3A, unless otherwise noted. However, by use of the above methods, results were obtained for

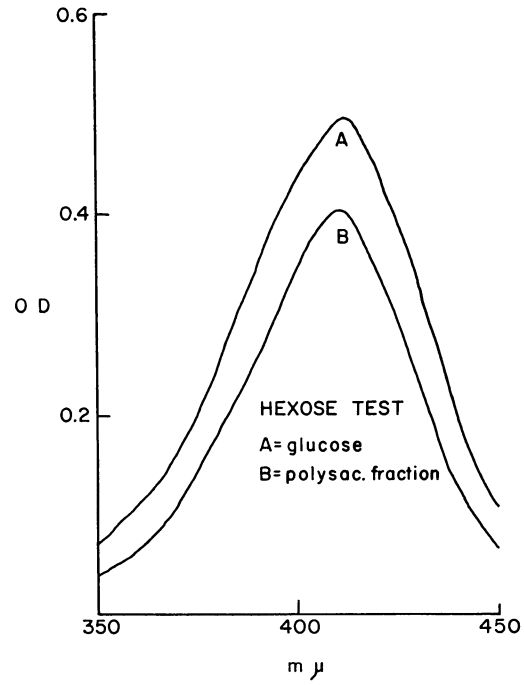

FIG. 5. *Absorption spectra for glucose, and a hexose isolated from the neutral fraction of a hydrolysate of extracellular polysaccharide from strain 3A, in the primary cysteine-sulfuric acid test for hexose.*

SP 102 and 155 identical to those obtained for SP 3A. Consequently, the polysaccharides of *A. vinelandii* under investigation had the four major components listed above in common.

Additional components of the polysaccharides not found in common are as follows. *O*-acetyl groups (Hestrin, 1949) were detected in SP 155 and 102, but not in SP 3A. Samples of SP 3A recovered from different culture batches, and at various stages of purification, were tested with similar negative results. Harsh treatment of SP 3A with heat or alkali was avoided during purification procedures to eliminate the likelihood of ester hydrolysis. These data confirm those obtained from infrared analyses of SP 155, 3A, and 102 (Fig. 1).

Within recent years, several polysaccharides of bacterial origin have been found to contain sialic acid (Barry, Chen, and Roark, 1963). Figure 6 illustrates the identical absorption spectra produced by SP 155 in the thiobarbituric acid assay (TBA) of Warren (1959) for sialic acid with that of authentic *N*-acetylneuraminic acid. A positive TBA reaction was not observed for either SP 3A or 102. Hydrolyzed (0.1 N H_2SO_4 for 1 hr at 80 C) or unhydrolyzed SP 155 yielded identical spectra with this test and, furthermore, showed approximately the same quantitative content (1.5% of the dry weight of the polysaccharide). These values were calculated according to Warren (1959) with *N*-acetyl neuraminic acid as a standard. To determine whether this component was free, SP 155 was exhaustively dialyzed against distilled water and 0.1 N H_2SO_4, but the total TBA reactivity remained with the polymer. So far, efforts to remove the TBA reactive component by the use of various hydrolyzing conditions (0.1 N or 1.0 N H_2SO_4 at 80 C, for varying periods of time) have been unsuccessful. Harsh conditions (i.e., 1.0 N H_2SO_4 at 100 C) resulted in the loss of reactivity in the TBA assay. Therefore, this moiety appeared not to be bound by the acid-labile ketosidic linkage usually found with molecules containing sialic acid. Sialic acid was not detected by the modified Ehrlich reaction (Barry, Abbott, and Tsai, 1962), with the use of 15 mg of SP 155, which contained approximately 200 μg of the TBA-reactive compound. Barry, Hamm, and Graham (1963) recently stated that sialic acid is strongly indicated in bacteria which give a positive Ehrlich test, but not in those which

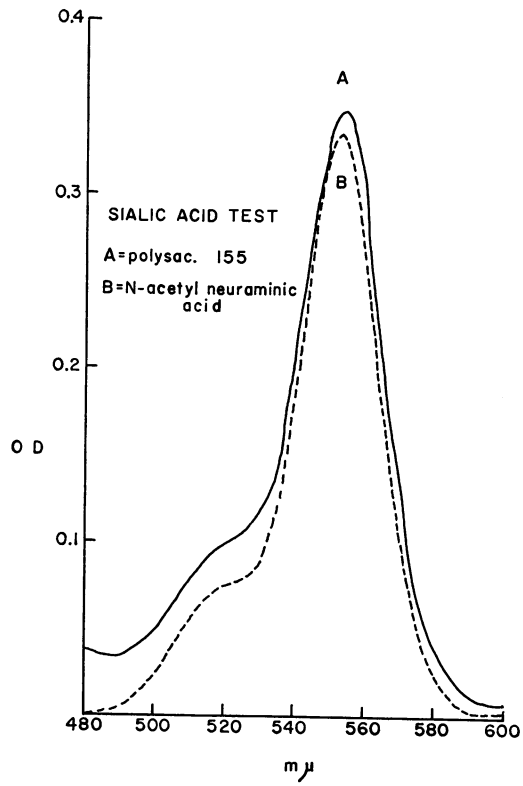

FIG. 6. *Absorption spectra for intact extracellular polysaccharide of strain 155, and N-acetyl neuraminic acid, in the thiobarbituric acid assay for sialic acid.*

give only a positive Warren test. Furthermore, since the thiobarbituric acid chromaphores of sialic acids and 2-keto-3-deoxy sugar acids behave similarly (Warren, 1959), further studies are now in progress to determine the conditions for hydrolysis of the component. The TBA-reactive component may then be determined by methods other than colorimetric.

Capsular polysaccharide of strain 3A was separated from the slime, purified, and analyzed by the above methods. Results showed that capsular and slime polysaccharide were chemically identical in composition. When glucose, fructose, or ethanol was used as the sole carbon and energy source, no change was observed in the composition of SP 3A.

Table 2 shows representative results of quantitative analyses for SP based on dry weight of the polymer and adjusted for ash and protein content. The results obtained for glucose and rhamnose content represent both colorimetric

TABLE 2. *Chemical analyses of extracellular polysaccharides of Azotobacter vinelandii*[a]

Strain	Nitrogen[b]	Carbohydrate[b]	Galacturonic acid[b]	Glucose[b,c]	Rhamnose[b,c]	*O*-acetyl[b]	Ratio[d]
	%	%	%	%	%	%	
3A	0.14	50.5	41.1	1.2	1.4	Not detected	41:1:1
102	0.76	45.5	37.9	1.5	1.7	2.9	38:2:2
155	0.38	46.0	49.5	4.1	0.9	6.5	50:4:1

[a] All figures are calculated on a dry weight basis adjusted for ash and protein.
[b] Analysis performed on intact polysaccharides.
[c] Analysis performed on acid hydrolysates
[d] Per cent ratio of galacturonic acid–glucose–rhamnose.

analyses on the intact polymer and quantitative paper chromatography, since these methods yielded virtually identical results.

Attempts were made to account for the remainder of the dry weight of SP. Various conditions for acid hydrolysis were employed in conjunction with sugar detection methods; however, only the four reported components were observed. Hexosamine was not found in the polysaccharides investigated. Furthermore, Kjeldahl nitrogen in SP was accounted for as protein by the method of Lowry et al. (1951).

DISCUSSION

These studies confirm, in part, the results of Kaufman and Rapaske (1958), and Kaufman (1960), who indicated that the capsular polysaccharide consisted of glucose and rhamnose units. The evidence reported here, however, indicates that galacturonic acid is also present and is a major component of the polysaccharide. In addition, a hexuronic acid lactone, probably mannurono-lactone, is also present in acid hydrolysates. Whereas Kaufman (1960) reported that capsules and slime possess different chemical composition, the present study shows them to possess the same four major components. This is consistent with Wilkinson's (1958) statement that if cells produce both capsules and slime, they are of similar composition. Abovementioned differences may have been due in part to the resistance of the polysaccharide to acid hydrolysis. For example, after 5 hr of hydrolysis with 2 N H_2SO_4, only traces of uronic acid were detected by paper chromatography. However, colorimetric methods of Dische for sugar analysis on intact polysaccharide provided evidence for the composition of *A. vinelandii* polymers not easily detected by paper chromatography.

In addition to the four major components common to the strains, *o*-acetyl was found in only two of the three strains, one of which also contained a thiobarbituric acid assay (Warren, 1959)-positive component. Therefore, it appears that the polysaccharides are similar in many respects, but are not identical.

Although evidence suggests that the lactone is mannurono-lactone, further studies are needed for confirmation. The lactone resembles the unknown component, X1, described by Zaitseva, Belozerskii, and Afanas'eva (1959), who studied the gross polysaccharide content of *A. vinelandii*. If, indeed, two uronic acid residues are present, it would represent one of the few such reports for bacterial extracellular polysaccharides. *Aerobacter aerogenes* type 64 extracellular polysaccharide was found to contain glucuronic and mannuronic acid (Barker et al., 1958).

Two components, glucose and rhamnose, were found in common with cyst polysaccharide (Wyss et al., 1961). Further work is needed to determine whether cyst polysaccharide does, in fact, contain all of the components shown here in extracellular polysaccharide of the vegetative cell.

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