Colonial variation in *Xanthomonas campestris* NRRL B-1459 and characterization of the polysaccharide from a variant strain[1]

M. C. CADMUS, S. P. ROGOVIN, K. A. BURTON, J. E. PITTSLEY, C. A. KNUTSON, AND ALLENE JEANES

Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL, U.S.A. 61604

Accepted March 19, 1976

CADMUS, M. C., S. P. ROGOVIN, K. A. BURTON, J. E. PITTSLEY, C. A. KNUTSON, and A. JEANES. 1976. Colonial variation in *Xanthomonas campestris* NRRL B-1459 and characterization of the polysaccharide from a variant strain. Can. J. Microbiol. **22**: 942–948.

Stock cultures of *Xanthomonas campestris* NRRL B-1459 require special attention to maintenance and propagation to assure consistent production in good yields of the extracellular polysaccharide xanthan. Under customary conditions of propagative maintenance on agar slants, variant colony types develop that are smaller in size than the normal type. The rate of regression of the normal to the variant forms was diminished when the D-glucose content of the stock medium was sufficient to avoid depletion during storage and when transfer to fresh medium was reduced to 14-day intervals. Under conditions for polysaccharide production, the normal large-colony type gives crude culture liquors after 48 h of 7000 centipoise (cp) viscosity; the predominant variant form gives only 4000 cp. On the basis of 2.1% initial D-glucose, biopolymer yields for the normal and variant strains were 62 and 43%, respectively. Polysaccharide produced by the variant (small-colony type) differs adversely in solution properties from that of the parent strain (large-colony type); it differs also in its lower content of pyruvic acid and *O*-acetyl substituents. The molar ratios of constituent sugars (D-glucose, D-mannose, and D-glucuronic acid), however, were identical in polysaccharides with the normal and variant strains. Exclusion of colonial variants from fermentations is prerequisite to production of xanthan optimum in properties and yield.

CADMUS, M. C., S. P. ROGOVIN, K. A. BURTON, J. E. PITTSLEY, C. A. KNUTSON et A. JEANES. 1976. Colonial variation in *Xanthomonas campestris* NRRL B-1459 and characterization of the polysaccharide from a variant strain. Can. J. Microbiol. **22**: 942–948.

L'entreposage de cultures de *Xanthomonas campestris* NRRL B-1459 requiert une attention spéciale de maintien et de propagation pour assurer une production constante de bons rendements en xanthane, polysaccharide extracellulaire. Dans les conditions habituelles de maintien de la propagation sur agars penchés, des colonies types de variants se développent dont les dimensions sont inférieures à celles du type normal. Le taux de régression des formes normales vers les variantes diminue si la teneur en D-glucose du milieu d'entreposage est suffisante pour prévenir son épuisement au cours de l'entreposage et si les intervalles de transfert à des milieux frais sont réduits à 14 jours. Dans les conditions de production du polysaccharide, le type normal de grande colonie produit, après 48 h, des liqueurs brutes de culture de 7000 'centipoise' (cp) de viscosité; la forme variante prédominante ne fournit que 4000 cp. Sur la base de 2.1% de D-glucose initial, les rendements en biopolymères pour les souches normales et les variantes sont respectivement de 62 et de 43%. Le polysaccharide produit par le variant (type petite colonie) diffère de façon adverse dans ses propriétés de solution de celui de la souche parentale (type grande colonie); il diffère aussi en une teneur inférieure en acide pyruvique et en substituts *O*-acétyl. Les rapports molaires des constituants en sucres (D-glucose, D-mannose, et acide D-glucuronique) sont cependant identiques en polysaccharides tant pour les souches normales que pour les variantes. L'exclusion des colonies variantes aux fins de fermentations est un prérequis pour la production optimum en propriétés et en rendement de xanthane.

[Traduit par le journal]

Introduction

The exocellular anionic heteropolysaccharide (xanthan) from *Xanthomonas campestris* NRRL[2] B-1459, now produced industrially in both the United States (11) and Europe (6), has numerous applications in food and nonfood industries (8).

This hydrophilic colloid has advantages over colloids of land plant origin because by controlled fermentation a product of constant and reproducible quality and properties results. However, the physiological state of the strain and its synthetic activity must be maintained constant and invariable.

[1] Received October 6, 1975.

[2] NRRL, Northern Regional Research Laboratory.

Purchased by U.S. Department of Agriculture for official use.

Scientists (16, 17) at the Northern Laboratory have established microbial and bioengineering procedures that favor reproducible fermentations with this strain and batch-type production of its biopolymer. Under conditions of continuous fermentation, however, development of variant forms and eventual cessation of polysaccharide production have been observed (18). It now has been found that variant colony forms develop also during maintenance of the culture under certain propagative conditions and that one variant strain produces polysaccharide in lower yield and differing in some aspects of composition and properties from the polysaccharide originally reported (10) for the normal strain.

In view of the increasing industrial, food, and academic usage of xanthan gum, procedures for maintenance and propagation of *X. campestris* B-1459 must be established that assure a homogeneous population of cells which produce polysaccharide of high quality in optimum yields.

Materials and Methods

Media

Media for cultivation of stock cultures were yeast malt (YM) agar (7), tryptone–glucose–yeast (TGY) agar (7), and T2GY (TGY containing 0.2 to 1.0% D-glucose) agar; YM broth was also used for propagation of cells. Production medium contained 2.1% D-glucose (sterilized separately), 0.8% distillers' dried solubles, 0.5% K_2HPO_4, and 0.01% $MgSO_4$ in tap water and was adjusted to pH 7.0.

Maintenance

Strains of NRRL B-1459 were preserved by lyophilization (7). To initiate growth, the contents of a lyophile tube were transferred aseptically to, and suspended in, 7 ml of sterile YM broth and incubated for 24 h on a shaker. This broth culture was transferred onto YM agar slants, incubated for 24 h at 25 °C, and stored at 4 °C; purity (large colonies of uniform size and color) was determined by streaking YM agar plates and incubating them for 3 days at 25 to 28 °C. Stock cultures were transferred (small loop) to fresh slant medium (less than 1 month old) every 14 days and incubated no longer than 18 to 20 h at 25 °C. Optimum biopolymer production was assured by starting agar-slant cultures from a fresh lyophile culture every 3 months.

Gum Productivity

Conditions for production of xanthan gum on laboratory and pilot-plant scales have been reported before (15–17). For our study we used the following method to test the productivity of a particular strain of *X. campestris*: a loopful of inoculum from a fresh stock culture (not more than 3 days old) was transferred to 7 ml of sterile YM broth contained in a 18 × 150 mm tube (first stage) and incubated at a 25° inclination on either a reciprocal shaker (100 strokes/min; 6.3 cm each) or a rotary shaker (160 rpm; 7.5 cm eccentric) for 24 h at 28 °C. The first-stage culture was added to 43 ml of YM broth in a 300-ml Erlenmeyer flask, and this second stage was incubated 18 to 24 h on the rotary shaker. For the third and last stage, 500 ml of production medium, contained in a 2800-ml Fernbach flask with a gauze-covered cap (four 6-in.-diameter gauze-faced disks (milk filters)), was inoculated at the rate of 5% v/v and incubated on the rotary shaker (200 rpm) for 48 h. After water was added to compensate for evaporation loss (about 2%), viscosity was measured with a Brookfield[3] model LVF viscometer at 30 rpm and 25 °C. A good fermentation developed viscosity greater than 6800 centipoise (cp).

Recovery of Product

Polysaccharide B-1459 has been recovered by several precipitation and drying procedures on both laboratory (10) and pilot-plant scales (1, 15, 16). In our work, we used the following procedure to recover purified xanthan suitable for analysis: crude culture liquor, prepared as described, was diluted with distilled water and ethanol (up to 40% v/v) while stirring rapidly until the viscosity was about 300 cp. Most of the cells and other debris were removed by continuous centrifugation at 50 000 rpm (Sharples type T-1, Sharples-Stokes Co., Warminster, Pennsylvania). Gum was precipitated by the addition of first 1% KCl (based on water equivalent) and then ethanol to 55% (v/v). Precipitated gum was separated from the supernatant fluid on a stainless-steel sieve. Polysaccharide was redissolved in sufficient water (and alcohol to 40% v/v) to attain a viscosity of 40 to 50 cp, centrifuged to remove additional cells, and reprecipitated. The product was redissolved again in water to about 500 cp, reprecipitated, and dissolved in water to about 300 cp. The solution was dialyzed against distilled water (25 °C) until either the conductivity of the water was constant or pH of the gum solution was about 4.5. The solution was adjusted to pH 6.2 with 1 *M* KOH, passed through a sintered glass filter (medium, Pyrex No. 36060), concentrated, and lyophilized.

Isolation and Characteristics of a Variant Strain

Initially, cultures of B-1459 were maintained on either YM or TGY agar, transferred to fresh medium at least once a week, and incubated for 24 h at 25 °C. After 12 to 18 months, these cultures gave significantly lower yields of polysaccharide. When they were streaked on YM agar plates and incubated for 72 h at 25 °C, two distinct colony types arose as evidenced by size and pigmentation. Large (L) (4 to 5 mm diam) light yellow mucoid colonies and small (S_m) (about 2 mm diam) darker yellow mucoid colonies were about equal in number. Very small nonmucoid colonies (about 1 mm diam) also were observed occasionally. Subsequent plating of L colonies yielded 98 to 100% of the L type; sequential plating and reisolation resulted in L strains that produced colonies of uniform size and color when streaked on YM plates. When carried through batch fermentations for polysaccharide production, the selected L strains did not revert to mixtures of L and S_m colony types.

S_m colonies, when isolated from YM agar plates

[3]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

streaked with the aged stock culture and replated, yielded from 50 to 100% of the S_m type. Upon sequential plating, reisolation, and restreaking, S_m-type colonies appeared to produce only small colonies and, when carried through a batch fermentation for polysaccharide production, a selected S_m strain (S_m2) retained its distinctive characteristics. However, the observation stated above that at least certain S_m colonies can revert to the L type, accords with a previous report (18).

Analytical Measurements

Inorganic contents of purified polysaccharide were determined on dry samples by standard microanalytical methods: (i) nitrogen, by the Kjeldahl procedure (22); (ii) phosphorus, gravimetrically as ammonium phosphomolybdate (22); and (iii) ash, as the sulfate of cations present, by adding several drops of 9 *N* H_2SO_4 to the sample and igniting at 600 °C, adding several drops of both 9 *N* H_2SO_4 and 7.5 *N* HNO_3 and ashing again (microburner), and repeating the last treatment if traces of carbon remain.

When plentiful volumes of solution were available for viscosity measurement, such as crude culture liquors and their dilutions undergoing purification, a Brookfield viscometer model LVF (Brookfield Engineering Laboratories, Inc., Stoughton, Massachusetts, U.S.A.) was used at 30 rpm and 25 °C.

For characterization of purified polysaccharide solutions, however, viscosity was measured with the Wells-Brookfield micro viscometer (cone and plate, model RVT) at 3.84 s^{-1} (1 rpm) and 25 °C. (The practical equivalence of values obtained with these two viscometers under the conditions stated has been established (9).) Intrinsic viscosity in 0.01 *M* ammonium acetate was measured at 25.0 °C in size 75 Cannon semimicro viscometers (Cannon Instrument Company, State College, Pennsylvania, U.S.A.).

Solutions containing a minimum of extraneous ionic matter were used to determine the effect of pH on viscosity. Test solutions were made by judicious admixture, so as to maintain constant polysaccharide concentration, of two solutions: (i) consisting of the free-carboxyl form of the polysaccharide obtained by decationizing a 0.5% solution of the polysaccharide (neutral K-salt form) on a column of AG 50W-X4 resin (20 to 50 mesh, H^+ form, Bio-Rad Laboratories) and (ii) having a final concentration of 0.5% polysaccharide, made by adjusting the pH of a solution of the polysaccharide (neutral K-salt form) to pH 11 to 12 with dilute KOH. The pH of the decationized solution is 2.20 ± 0.02; acetic acid is added to obtain lower values.

The ratio of component sugars in xanthan was determined by radiochromatographic analysis of an acid hydrolyzate after reduction with ^{3}H-sodium borohydride (12).

Pyruvic acid was measured in an acid hydrolyzate of xanthan by an enzymic procedure (4).

O-acetyl was determined by the hydroxamic acid method (14).

Results

Maintenance of L-type Culture

The stability of stock cultures of strain NRRL B-1459, L type, as indicated by the rate and extent of regression to the S_m variant, is influenced by both the frequency of transfer and the composition of the media. Biweekly transfer to fresh slant media resulted in best stability. More frequent transfers led to rapid regression to the S_m form. Regression was slowest on YM agar (Table 1). For biweekly transfer, YM was markedly superior to TGY and even at the unfavorable weekly transfer rate, YM was superior to T2GY. The low sugar level (0.1%) of TGY led to culture deterioration within 10 months (Table 1) whereas D-glucose levels of 0.2 to 1.0% in T2GY afforded improved stability. Cells that form small colonies have been found, however, in inocula grown from stock maintained on T2GY agar and transferred biweekly for no longer than 3 months (18).

TABLE 1. Effect of culture-transfer frequency on colony-size ratio with *Xanthomonas campestris* NRRL B-1459

No. transfers/14 days	Agar slants used[a]	Ratio L:S_m[b] colonies at 10 months
1	TGY	47:1
2	TGY	10:1
6	TGY	1:3
2	T2GY	50:1
1	YM	200:0
2	YM	120:1
6	YM	1:1

[a]All cultures at zero time were composed of uniformly large colonies after plating. Agar abbreviations: TGY = tryptone–glucose–yeast (0.1% D-glucose); T2GY = TGY containing from 0.2 to 1.0% D-glucose; YM = yeast malt (1.0% D-glucose).
[b]L = large colony; S_m = small mucoid colony.

After 14 days' storage of cultures on agar slants, viable cell population diminishes rapidly. Maintenance on TGY agar containing less than 0.2% D-glucose reduced the number of viable cells to zero in only 28 days, whereas higher sugar levels preserved some viability up to 42 days.

It has not been established whether the S_m type shows stability comparable to the L type when maintained under conditions that are the best for the L type. Reversion of S_m to L appears to occur but the factors influencing this change are not known.

Polysaccharides of L and S_m-variant Strains

Yield and quality of product were tested for typical L (B-1459-L6) and S_m (B-1459-S_m2) strains. After 48 h of incubation in production medium, crude culture from strain L gave viscosities of 7000 cp, whereas the S_m culture gave

TABLE 2. Nitrogen, phosphorus, and ash values for B-1459 polysaccharides

Type, PS	Nitrogen,[a] g/100 g	Phosphorus,[b] g/100 g	Ash (sulfated),[c] g/100 g
PS-L	0.45	0.20	13.5±1.0
PS-S_m	0.35	0.15	10.0±1.0

NOTE: PS, polysaccharide.
[a]Residual protein from medium and bacterial cells.
[b]Residual phosphate from the medium.
[c]If all extraneous ions have been removed by dialysis, ash results exclusively from potassium ions that neutralize the glucuronic and pyruvic acids in the polysaccharides. The theoretical sulfated ash for the neutral potassium salt of xanthan products having glucuronic and pyruvic acid contents as reported here are 12.6% for PS-L and PS-P types and 11.0% for PS-S_m.

4000 cp. Polysaccharide yields, based on initial D-glucose concentration, were 62 and 43% from strains L and S_m, respectively. No significant differences were noted in viable cell counts of the growing cultures.

Characteristics of polysaccharides (PS) isolated from the large (PS-L) and small (PS-S_m) colony strains were compared, along with the biopolymer isolated previously from a parent strain (PS-P). Meaningful differences were observed in the nature of the precipitates obtained when the polysaccharides were precipitated from stirred aqueous solutions containing 1% KCl by adding ethanol to 55% concentration. PS-P and PS-L separate as cohesive strings of flocculent particles which tend to wind around the stirrer. In contrast, PS-S_m forms short noncohesive particles which are nearly transparent until excess alcohol is added.

The slightly lower values of nitrogen, phosphorus, and ash for PS-S_m than for PS-L (Table 2) are believed to reflect merely the relative ease of purification.

Viscosity measurements on the three purified polysaccharides, when observed in our usual way in the concentration range 0.1% to 2.0% (8, 10), revealed apparently minor differences in the shear ranges available in the micro cone-and-plate viscometer. For the three samples, viscosities at 0.5% concentration were in the range of 1000 cp to 1800 cp and at 1% the range was about 2900 cp to 3500 cp. Significant differences do exist, however, as shown by the intrinsic viscosity values $[\eta] = 34$ for PS-L and $[\eta] = 23$ for PS-S_m (measured in 0.01 *M* ammonium acetate at 25.0 °C and expressed in dcl/g).

Differences in solutions of polysaccharides PS-L, PS-S_m, and PS-P are shown also by the effect of salt concentrations on viscosity (Fig. 1).

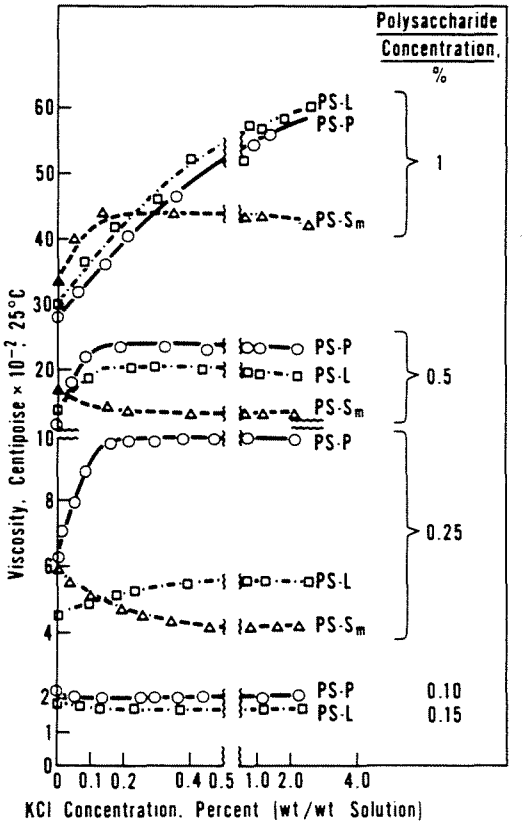

FIG. 1. Effect of salt (KCl) concentration on viscosity of 0.25, 0.5, and 1% solutions of polysaccharides PS-L, PS-S_m, and PS-P from B-1459 strains. Solid salt was added incrementally to each aqueous solution.

Apparently, PS-L resembles PS-P, especially at the higher polysaccharide levels, whereas PS-S_m exhibits a different viscosity pattern in the presence of KCl, since it gives lower viscosities at the higher salt levels than do the corresponding concentrations of PS-L and PS-P.

The viscosities of salt-free solutions of the biopolymers (0.5%) isolated from L and S_m colony strains also responded differently to changes in pH (Fig. 2). For both products, the viscosity was essentially constant between about pH 5.0 and 8.5. As pH decreased to about 2.0 or increased to about 9.5, however, viscosity of PS-S_m decreased, whereas that of PS-L increased. The originally reported polysaccharide (PS-P) from NRRL B-1459 (10), which is also shown in Fig. 2, behaves similarly to PS-L.

The rheological properties of PS-S_m resemble those of PS-L and PS-P in being of the plastic type and having a moderately high working yield value (PS-S_m: 17 dynes/cm^2; PS-L: 21),

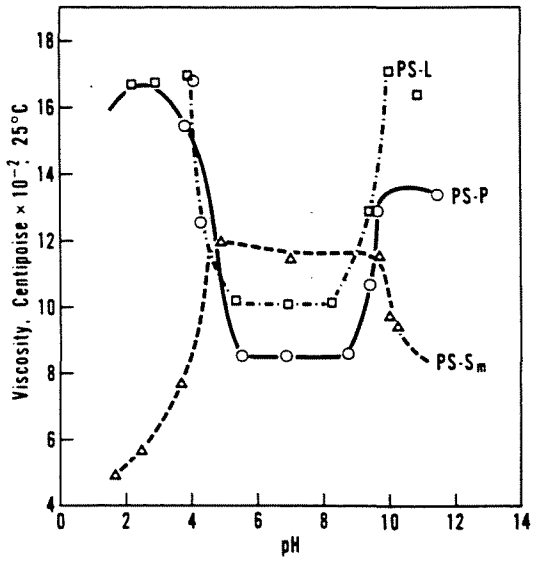

FIG. 2. Viscosity of B-1459 polysaccharide solutions PS-L, PS-S_m, and PS-P at 0.5% concentration over a pH range from 2 to 12.

and a low thixotropic index (PS-S_m: 1.0; PS-L: 1.2) (9). These measurements, made under specially defined conditions within the shear rate of 100 to 0 s^{-1}, show that solutions of these polysaccharides thin rapidly and reversibly under low shear and may be expected to have good suspending properties.

Chemical analyses on PS-L and PS-S_m made by a highly sensitive method (12) showed both to have the same proportion of component sugars. However, Table 3 reveals differences in *O*-acetyl and pyruvic acid contents. Pyruvic acid values for PS-L and PS-P are similar, whereas the level in PS-S_m is significantly less. The acetyl value for PS-S_m is also lower than for PS-L.

TABLE 3. Acetyl and pyruvic acid contents of B-1459 polysaccharides

Type PS	*O*-acetyl, g/100 g	Pyruvic, g/100 g
PS-L	4.5	4.4
PS-S_m	3.7	2.5
PS-P[a]	4.7	4.7

[a]P = parent (also large-colony type).

Discussion

Variation in the Culture

Variation is a recognized characteristic in *Xanthomonas* species (21). A series of colonial variants in *X. phaseoli* has been described (2). Each type produced polysaccharide; the amount of polysaccharide determined the colony type, correlated with the virulence, and was genetically controlled (3). Serological tests suggested that the same or antigenically similar polysaccharides occurred in the four variants. In a strain of *X. campestris*, isolated initially as a single cell and maintained on nutritive agar, a few small colonies were found among the normal large mucoid colonies (23). The small colony isolate, considered a possible mutant, produced less polysaccharide and was less virulent than the large colony strain (24).

In *X. campestris* NRRL B-1459, colonial variants now have been shown to develop under certain conditions of culture maintenance on agar media. Previously, under conditions of continuous fermentation, this strain has shown colonial variation (18) or bacterial dissociation (13). In both culture maintenance and continuous fermentation, the normal large-colony type, which is a vigorous producer of polysaccharide, was displaced by a smaller more pigmented type that produces less polysaccharide. In research on continuous fermentation, the inocula for which consisted of at least 90% L-type cells, steady states were achieved through 6.5 to 8.7 turnovers when a small-type variant became dominant, steady states ended, and polysaccharide production ceased (18). Standard batch fermentations, run as controls on the inocula for the continuous fermentations, proceeded normally without interference by a variant.

The causes of culture deterioration during continuous fermentation are not known and will require systematic investigation to resolve. However, the unfavorable effects resulting from inadequate glucose concentrations and too frequent transfer on culture stability during stock maintenance may be effective, also, in continuous fermentation.

Use of propagative conditions for maintaining working stock cultures of normal L-type B-1459 strains that are relatively free of variant forms (Table 1) has two prerequisites: (i) using a stock slant medium containing sufficient D-glucose to meet the need of the culture without depletion during storage and (ii) limiting transfer to fresh media to once every 2 weeks. Slow metabolism occurs under these conditions, however, and

development of variant forms is not completely repressed. An additional prerequisite necessary at intervals of no longer than 3 months is, therefore, to initiate a new series of agar-slant cultures from a fresh lyophil culture.

To preclude genetic change during short-term as well as long-term storage, nonpropagative conditions are widely recognized as necessary. Nonpropagative procedures designed to be more generally practical than lyophilization for short-term storage have been developed and shown to prevent reversion from normal large colonies to variant-size forms in working stock cultures of NRRL B-1459 (Alberta I. Herman (Fermentation Laboratory, Northern Regional Research Laboratory), personal communication; Denis K. Kidby (Department of Microbiology, University of Guelph, Guelph, Ontario, Canada), in preparation for publication in Applied Microbiology).

Adherence to these principles of culture maintenance result in inocula consisting predominantly of L-type cells which will produce high-quality xanthan in good yields under the established conditions of batch fermentation (16, 17). Nutritive and environmental requirements already established as basic for xanthan production are inoculum size, suitable nitrogen and mineral sources, pH, temperature, aeration, and length of incubation (16, 17).

Polysaccharides of B-1459: Comparison of PS-L and PS-S_m2

These polysaccharides differ in yield, some aspects of composition, and some significant properties. The variant strain produces only about 70% as much polysaccharide (PS-S_m) as does the normal (PS-L). Identical molar ratios of component sugars D-glucose : D-mannose : D-glucuronic acid (2:2:1) determined for both polysaccharides agree with that of the control (PS-P) (12, 19). The pyruvic acid substituent of PS-S_m, however, is only 56% that of PS-L and 53% of the polysaccharide control (Table 3). The significantly lower content in PS-S_m of the quite strongly ionized pyruvic acid may be expected to influence appreciably the polyelectrolyte character of PS-S_m. The content of the *O*-acetyl substituent is also lower in PS-S_m than in both PS-L and the control. Identical ratios of component sugars in PS-S_m and PS-L, however, indicate that the basic structures must be the same.

The diminished content of pyruvic acid in PS-S_m may provide a clue to the weak virulence of small-colony variants of other phytopathogens (2, 24). Pyruvic acid, which occurs in xanthan (20) and numerous other bacterial polysaccharides as a 4,6-*O*-(1-carboxy-ethylidene)-D-hexose residue, is an immunologic determinant in *Rhizobia* (5) and certain other capsulated bacteria.

Differences observed in the nature of alcohol precipitates of PS-L and PS-S_m are not fortuitous but are based on chemical constitution and resultant factors such as water-binding capacity, macromolecular dimensions, and conformation. Molecular weight may also be contributory. The lower intrinsic viscosity of PS-S_m as compared with that of PS-L is likely to be an indication of difference in molecular weight.

Establishment of the rheological profile through measurement of shear stress vs. shear rate relationships (9) is not sufficiently sensitive to reveal the extent of difference that seems to exist between PS-S_m and PS-L. The working yield value of PS-S_m (17 dynes/cm^2), although lower than PS-L (21 dynes/cm^2), is within the range of 12 to 32 dynes/cm^2 observed for numerous PS-P preparations.

When an electrolyte, such as salt (KCl) is added to xanthan solutions, the resultant change in viscosity will differ with the polysaccharide concentration. At 1% polysaccharide, PS-L resembles closely the usual PS-P behavior (10) (Fig. 2), but PS-S_m differs in the lesser augmentation of viscosity at KCl concentrations above 0.2%. The decreased viscosity with salt shown by PS-S_m at 0.5% concentration contrasts with PS-L and the usual PS-P preparations, which decrease in viscosity only at levels of 0.25% and lower. If admixed with PS-L, PS-S_m polysaccharide would adversely affect the viscosity of dilute solutions in numerous practical applications.

Additional indication of difference between PS-S_m and PS-L/PS-P resides in contrasting influence of pH on viscosity in the ranges between pH 3 to 5 and pH 9 to 10 (Fig. 2). It remains to be established how the magnitude and distribution of ionic charge may affect this behavior.

Data reported here establish the adverse effects of colonial variants on both the yield and properties of xanthan. Further research is necessary, however, to define more exactly the differences between the products of the normal and variant strains and the underlying molecular bases.

Information on structural differences of the polysaccharides from the normal and variant strains, if established, would contribute to understanding the mechanism of xanthan synthesis and the physical and structural features that determine the unusual properties of xanthan.

Acknowledgments

We thank Dr. A. I. Herman of the Northern Laboratory for her advice on strains of B-1459, Dr. F. R. Dintzis for intrinsic viscosity measurements, and Mrs. C. E. McGrew for nitrogen, phosphorous, and ash analyses.

1. ALBRECHT, W. J., V. E. SOHNS, and S. P. ROGOVIN. 1963. Pilot-plant process for the isolation of a microbial polysaccharide with a quaternary ammonium compound. Biotechnol. Bioeng. **5**: 91–99.
2. COREY, R. R., and M. P. STARR. 1957. Colony types of *Xanthomonas phaseoli*. J. Bacteriol. **74**: 137–140.
3. COREY, R. R., and M. P. STARR. 1957. Genetic transformation of colony type in *Xanthomonas phaseoli*. J. Bacteriol. **74**: 141–145.
4. DUCKWORTH, M., and W. YAPHE. 1970. Definitive assay for pyruvic acid in agar and other algal polysaccharides. Chem. Ind. (N.Y.): 747.
5. DUDMAN, W. F., and M. HEIDELBERGER. 1969. Immuno-chemistry of newly found substituents of polysaccharides of *Rhizobium* species. Science, **164**: 954–955.
6. GODET, P. 1973. Fermentation of polysaccharide gums. Process Biochem. **8**: 33–34.
7. HAYNES, W. C., L. J. WICKERHAM, and C. W. HESSELTINE. 1955. Maintenance of cultures of industrially important microorganisms. Appl. Microbiol. **3**: 361–368.
8. JEANES, A. 1974. Applications of extracellular microbial polysaccharide-polyelectrolytes: Review of literature, including patents. J. Polym. Sci., Polym. Symp. No. 45, pp. 209–227.
9. JEANES, A., and J. E. PITTSLEY. 1973. Viscosity profiles for aqueous dispersions of extracellular anionic microbial polysaccharides. J. Appl. Polym. Sci. **17**: 1621–1624.
10. JEANES, A., J. E. PITTSLEY, and F. R. SENTI. 1961. Polysaccharide B-1459: a new hydrocolloid polyelectrolyte produced from glucose by bacterial fermentation. J. Appl. Polym. Sci. **5**: 519–526.
11. KELCO CO. 1972. Xanthan gum/Keltrol/Kelzan/ a natural biopolysaccharide for scientific water control. Kelco Co. Publ., San Diego, California 92123.
12. KNUTSON, C. A. 1975. Paper-chromatographic separation of ^{3}H borohydride-reduced hexoses for quantitative determination of polysaccharide composition. Carbohydr. Res. **43**: 225–231.
13. LINDBLOM, G. P., and J. T. PATTON (Assignors to Esso Production Research Company). 1967. Heteropolysaccharide fermentation process. U.S. Patent No. 3 328 262.
14. 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.
15. ROGOVIN, P., W. ALBRECHT, and V. SOHNS. 1965. Production of industrial-grade polysaccharide B-1459. Biotechnol. Bioeng. **7**: 161–169.
16. ROGOVIN, S. P., R. F. ANDERSON, and M. C. CADMUS. 1961. Production of polysaccharide with *Xanthomonas campestris*. J. Biochem. Microbiol. Technol. Eng. **3**: 51–63.
17. SILMAN, R. W., and P. ROGOVIN. 1970. Continuous fermentation to produce xanthan biopolymer: Laboratory investigation. Biotechnol. Bioeng. **12**: 75–83.
18. SILMAN, R. W., and P. ROGOVIN. 1972. Continuous fermentation to produce xanthan biopolymer: Effect of dilution rate. Biotechnol. Bioeng. **14**: 23–31.
19. SLONEKER, J. H., and A. JEANES. 1962. Exocellular bacterial polysaccharide from *Xanthomonas campestris* NRRL B-1459. Part I. Constitution. Can. J. Chem. **40**: 2066–2071.
20. SLONEKER, J. H., D. G. ORENTAS, and A. JEANES. 1964. Exocellular bacterial polysaccharide from *Xanthomonas campestris* NRRL B-1459. Part III. Structure. Can. J. Chem. **42**: 1261–1269.
21. STARR, M. P. 1959. Bacteria as plant pathogens. Annu. Rev. Microbiol. **13**: 211–238.
22. STEYERMARK, A. 1961. Quantitative organic microanalysis. 2nd ed. Chapters 8 and 12. Academic Press Inc., New York and London.
23. SUTTON, J. C., and P. H. WILLIAMS. 1970. Relation of xylem plugging to black rot lesion development in cabbage. Can. J. Bot. **48**: 391–401.
24. SUTTON, J. C., and P. H. WILLIAMS. 1970. Comparison of extracellular polysaccharide of *Xanthomonas campestris* from culture and from infected cabbage leaves. Can. J. Bot. **48**: 645–651.