APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1995, p. 1159–1162 Vol. 61, No. 3
0099-2240/95/$04.00+0

Studies on a Viscous, Gel-Forming Exopolysaccharide from *Sphingomonas paucimobilis* GS1

ANITA A. ASHTAPUTRE AND AVINASH K. SHAH*

Department of Microbiology and Biotechnology Centre, Faculty of Science, M. S. University of Baroda, Baroda-390 002, India

Received 18 August 1994/Accepted 5 December 1994

A new strain, *Sphingomonas paucimobilis* GS1, accumulated 6.5 g of a highly viscous exopolysaccharide per liter, using sucrose as a substrate. The anionic heteropolysaccharide contained the following, in grams per gram: glucose, 0.7; galacturonic acid, 0.11; glucuronic acid, 0.07; and acetate, 0.12. The viscosity of the exopolysaccharide (4.0 g/liter; 4,200 cP) was 5.5 times that of xanthan gum and was stable over a wide pH and temperature range as well as in the presence of NaCl. Deacetylated polymer produced a clear, agarlike, thermoreversible gel in the presence of cations. The gel strength of the modified polymer was four times that of agar and could withstand autoclaving.

Microbial polysaccharides have found a wide range of applications in food, pharmaceutical, petroleum, and other industries during the last three decades (17). The quality and supply of traditionally used plant- and seaweed-derived gums are affected by environmental factors such as seasonal variation and eutrophication (14). Microorganisms offer a more attractive alternative as they can be grown under controlled conditions and they greatly extend the range of available polymers because of their unique properties. The microbial exopolysaccharides (EPS) which have been commercially exploited include dextran from *Leuconostoc mesenteroides* (17) for use as a blood expander, xanthan gum from *Xanthomonas campestris* (6) as a viscosifier and stabilizer, alginate from *Pseudomonas* sp. (11) and *Azotobacter* sp. (4), gellan from *Aeromonas elodea* (12) and *Sphingomonas paucimobilis* (15), and curdlan from *Alcaligenes* sp. (8) as gelling agents, and pullulan from *Aureobasidium pullulans* (20) as a plastic material. There is still hope of developing new polysaccharides with properties superior to those of the existing polymers because of the diversity offered by microorganisms. In this paper we report the chemical and physical characterization of a new EPS obtained from a strain of *S. paucimobilis.*

The EPS-producing culture was isolated locally from soil on nitrogen-free sucrose agar medium and was sent for identification to the National Collection of Type Cultures, London, England, and the University of Nebraska Medical Center, Omaha. The organism was maintained and cultivated on a medium containing the following, in grams per liter: sucrose, 40; KNO_3, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; K_2HPO_4, 0.5; $CaSO_4$, 0.1; $NaMoO_4$, 0.05; and agar, 20; pH 7.2 ± 0.2. Cultivation was carried out in a 2.0-liter bioreactor (LH, Maidenhead, United Kingdom) containing 1.5 liters of medium at 28°C, with 1.0 vol/vol/min aeration and at pH 7.2 ± 0.2. Agitation was changed from 500 to 800 rpm late in the course of fermentation due to the increase in viscosity. After 72 h, the culture broth was diluted 30 times and centrifuged at 8,000 × *g* for 30 min (SS34 rotor, Sorvall RC5C; Dupont, Wilmington, Del.) to sediment the cells, and growth was measured gravimetrically. The polymer was precipitated from the supernatant with 3 volumes of acetone. It was subsequently dialyzed against water for 48 h, reprecipitated, and dried at 50°C and was estimated gravimetrically.

Deacetylation of the native polymer (2 g/liter) was carried out with NaOH (10 M) at pH 11.25 and 80°C for 5 min with vigorous stirring, followed by neutralization with concentrated HCl. The deacylated polymer was subsequently precipitated with 3 volumes of acetone. Uronic acid content was measured by the method of Dische (5). *O*-Acetyl and pyruvyl contents were measured by the methods of Hestrin (10) and Slonekar and Orentas (19), respectively. Sucrose content was estimated by the method of Handel (7). The monomeric composition of EPS was determined by high-performance liquid chromatography (HPLC) analysis of the hydrolyzed polymer. Hydrolysis of the polymer was carried out with 2 M H_2SO_4 at 100°C for 6 h, followed by neutralization with $CaCO_3$. The clear hydrolysate was analyzed on an HPLC (LC4A; Shimadzu, Tokyo, Japan), using a refractive index detector (RID 2AS; Shimadzu) and an SCR 101-N column (Shimadzu) with distilled water as the eluent, at 0.6 ml/min. Monomeric sugars were identified on the basis of retention time, using standard sugars. Molecular weight determination was accomplished by gel permeation chromatography on Ultrahydrogel columns 250 and 1000 (Waters, Milford, Mass.) in a series, using a Varian HPLC system (Varian, Sunnyvale, Calif.) with a refractive index detector (Varian IR3). Deionized water was used as the eluent, at 0.8 ml/min. Calibration was performed with dextran standards. Viscosity, shear stress, and shear rate were measured with a Brookfield viscometer (LVDV II+) and small-sample adapter, SSA-8R (Brookfield, Stoughton, Mass.), at spindle speeds of 5, 10, 20, 50, and 100 rpm at 30°C unless otherwise mentioned. The gels were aged for 15 h at 28 ± 2°C, and gel strength was measured with a Nikkansui type jelly tester (Kiya Seisakusho, Kyoto, Japan) at 28 ± 2°C. Sugars, dextran standards, and xanthan gum were obtained from Sigma Chemical Co., St. Louis, Mo. Agar was obtained from Difco Laboratories, Detroit, Mich. Other chemicals used were obtained locally.

The yellow-pigmented, EPS-producing culture was identified as *S. paucimobilis* GS1 (formerly *Pseudomonas paucimobilis*). The culture accumulated 6.5 g of EPS per liter, almost 75% of which was produced during exponential growth (up to 48 h); the remainder was accumulated in the stationary phase (Fig. 1a). The polymer was composed of 0.7 g of glucose, 0.11 g of galacturonic acid, and 0.07 g of glucuronic acid per g. Total

* Corresponding author. Mailing address: Department of Microbiology & Biotechnology Centre, Faculty of Science, M. S. University of Baroda, Baroda-390 002, India. Phone: 091-265-327796. Fax: 091-265-339231.

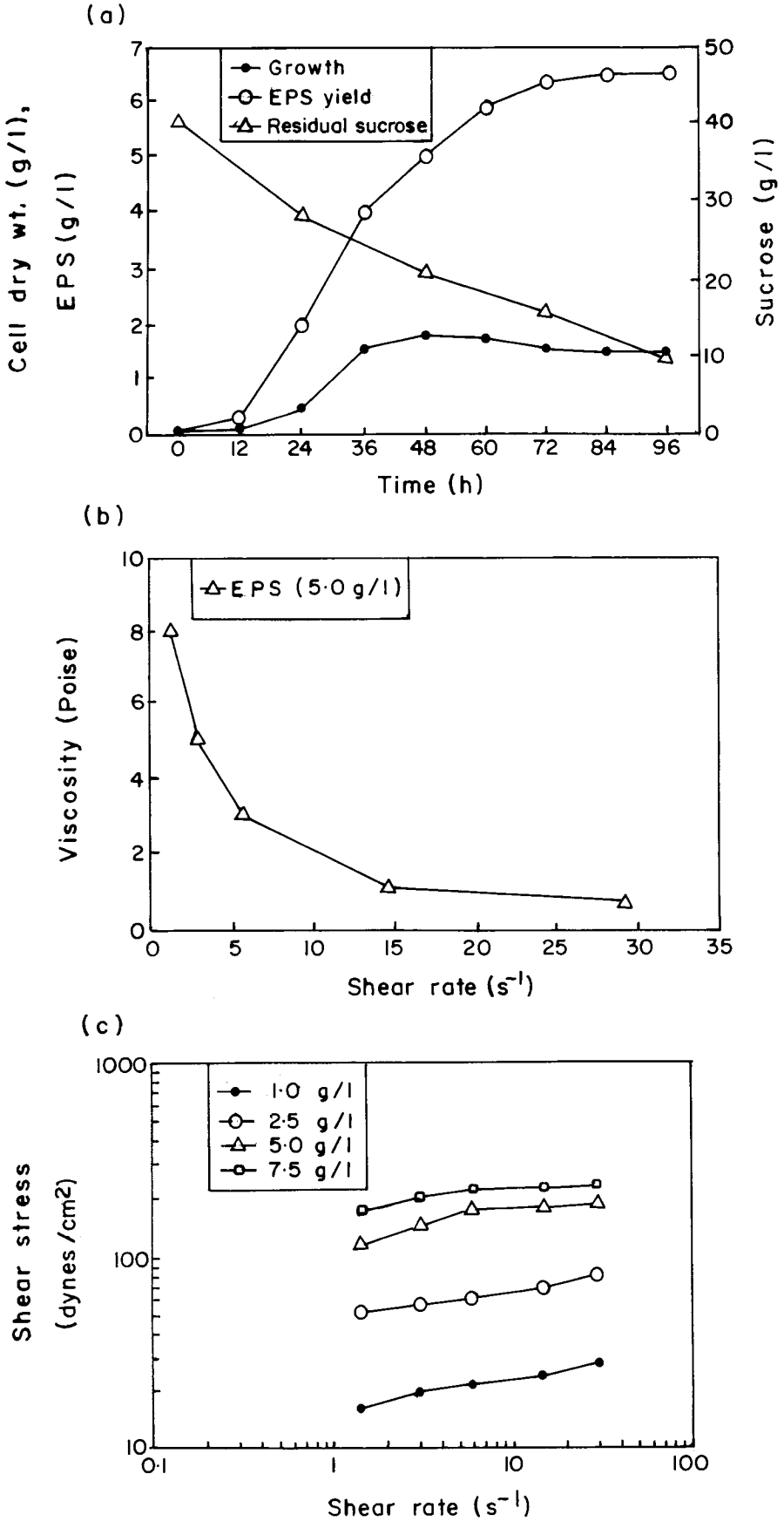
(a)
Growth
EPS yield
Residual sucrose
Cell dry wt. (g/l), EPS (g/l)
Sucrose (g/l)
Time (h)
(b)
EPS (5·0 g/l)
Viscosity (Poise)
Shear rate (s⁻¹)
(c)
1·0 g/l
2·5 g/l
5·0 g/l
7·5 g/l
Shear stress (dynes/cm²)
Shear rate (s⁻¹)

FIG. 1. Production and rheological properties of EPS from *S. paucimobilis* GS1. (a) Kinetics of EPS production. Cultivation was carried out in a 2.0-liter bioreactor at 28°C, with aeration of 1 vol/vol/min, a pH of 7.2 ± 0.2, and an agitation rate of 500 to 800 rpm. Growth, EPS, and sucrose were estimated at different time intervals. (b) Effect of shear on the viscosity of EPS. (c) Rheogram of aqueous solutions of EPS. EPS solutions (5.0 ml) were subjected to various shear rates, and the corresponding viscosity and shear stress values were measured with a viscometer at 30°C, as described in the text.

uronic acid, 0.15 g/g of EPS, was also detected from the unhydrolyzed polymer by colorimetric estimation. Acetate and pyruvate contents of 0.12 and 0.002 g/g, respectively, were also detected in EPS. The anionic nature of the EPS was determined by ion-exchange chromatography. Its molecular weight was in the range of 0.8×10^6 to 1.2×10^6, while that of xanthan gum, used for comparative studies, was 9×10^6 to 10×10^6.

EPS formed a viscous solution at a low concentration and the viscosity decreased with increasing shear rate (Fig. 1b). EPS (5.0 g/liter) had a yield point (minimum shear stress required for the fluid to flow) of 115 dynes/cm^2, while the consistency index, *K*, and flow index, *n*, obtained from the logarithmic plot of shear rate versus shear stress (Fig. 1c) were 101 dynes s^n/cm^2 and 0.35, respectively. Under identical conditions, the yield point, *K*, and *n* values obtained for xanthan gum were 21.5 dynes/cm^2, 25 dynes s^n/cm^2, and 0.40, respectively. The viscosity of EPS (4.0 g/liter) at 30°C and pH 7.0 was 5.5 times that of xanthan gum (4.0 g/liter) and remained unaffected up to 90°C, in the pH range 2 to 10, and also in the presence of NaCl up to 50 g/liter. The viscosity of xanthan gum was stable in the pH range 4 to 8, whereas only 26% of the original viscosity was retained at 90°C (Table 1). In the presence of NaCl (50 g/liter), the viscosity of xanthan gum (780 cP) was stable up to 90°C; nevertheless, the viscosity was less than that of EPS (4,150 cP). EPS viscosity was also not affected by other salts such as $CaCl_2$, $CoCl_2$, KCl, $MgCl_2$, $ZnCl_2$, $(NH_4)_2SO_4$, and NH_4Cl. The viscosity of EPS was superior to that of viscosifiers such as starch (50 g/liter; 600 cP), sodium alginate (20 g/liter; 480 cP), carboxymethyl cellulose (100 g/liter; 720 cP), and gum arabic (50 g/liter; 190 cP).

The polymer in its native form was incapable of forming a firm gel, but when deacylated, it formed a stiff, thermoreversible, clear gel in the presence of cations. Gelation could be obtained with K, Ca, Na, and Mg ions; however, NaCl was found to be most suitable. The melting and setting temperatures of a 10-g/liter gel induced with 5.0 g of NaCl per liter were 90 and 50°C, respectively. The setting temperature, however, varied between 45 and 50°C depending on the concentration of the polymer. The gel strength of deacylated EPS was four times that of agar and could withstand two cycles of autoclaving (Table 2). Normal growth of *Escherichia coli* was obtained on Luria-Bertani medium when deacylated EPS (10 g/liter) was used as a solidifying agent.

Polysaccharide production by *S. paucimobilis* GS1 followed a mixed kinetics pattern, which was similar to previous observations made for xanthan gum (14) and gellan from *S. paucimobilis* (15). EPS was anionic in nature due to the presence of uronic acids. Uronic acids have also been reported to be components of xanthan gum, alginic acid, gellan, and hyaluronic acid (13). However, the chemical composition of EPS from *S. paucimobilis* GS1 was different from that of these reported EPS (13, 18), indicating it to be a new polymer. The values of *K* and *n* have been determined according to the power law model (2). The *K* value obtained for EPS, which is a direct measure of the polymer viscosity, was almost five times that determined and reported for xanthan gum at identical concentrations (16). The viscosity of this EPS was also 5.5 times that of xanthan gum, although the molecular weight of EPS from *S. paucimobilis* GS1 was almost 10 times less than that of xanthan gum. This result is of significance as it has been reported for xanthan gum (9) and hyaluronic acid (1) that viscosity is greatly dependent on the molecular weight of the polymer. The flow index, *n*, is the measure of the pseudoplastic nature of a fluid, and the lower the value of *n*, the higher the psuedoplasticity (2). The *n* value of this EPS reflects its high pseudoplastic nature, which is one of the important properties for viscosity-based applications. Xanthan solutions were temperature stable only in the presence of NaCl. Deacylation of EPS probably facilitates greater cross-linking of the polymer chains, which results in the formation of stiff gels. Gel formation by xanthan-galactomannan is also favored by deacylation of xanthan gum (3). Similar observations have been made for gellan, which is currently the only polymer considered an agar substitute. The consistency of deacylated EPS gels was similar to that of agar. The gel strength of deacylated EPS gels was much higher than that of agar and somewhat greater than that reported for gellan (12). The melting-setting hysteresis of EPS was similar to that of gellan. Preliminary studies on the viscosity and gel-

TABLE 1. Viscosity of EPS from *S. paucimobilis* GS1 versus that of xanthan gum under various conditions

Parameter	Viscosity (cP)[a]	
	EPS (4.0 g/liter)	Xanthan gum (4.0 g/liter)
Temp (°C)[b]		
30	4,200	750
60	4,150	580
70	4,200	290
90	4,200	193
pH[c]		
2	4,100	365
4	4,120	780
6	4,150	775
8	4,200	780
10	4,200	680
NaCl (g/liter)		
0	4,180	740
25	4,205	760
50	4,210	780

[a] Viscosity was measured at a spindle speed of 10 rpm.
[b] Polymer solutions were maintained at the given temperature for 1 h.
[c] The pH of the solutions was adjusted with 1.0 N NaOH or 1.0 N HCl.

TABLE 2. Gel strength of deacylated EPS compared with that of agar

Cycles of autoclaving[a]	Deacylated EPS (10 g/liter)[b]		Agar (10 g/liter)	
	Gel strength (g/cm^2)	% Loss[c]	Gel strength (g/cm^2)	% Loss[c]
0	1,400		290	
1	1,385	1.08	240	17
2	1,385	1.08	230	20

[a] Autoclaving was done at 121°C for 15 min.
[b] Gelling was induced with NaCl (5.0 g/liter).
[c] Change in gel strength compared with autoclaving cycle 0.

ling properties of EPS reported here suggest its potential for industrial application.

We thank P. V. Thakore and N. Sheikh, R & D Centre, Gujarat State Fertilizer Co., Baroda, India, for making available the HPLC facilities for molecular weight determination of the polysaccharide samples and the Department of Biotechnology, India, for partial financial assistance. A. A. Ashtaputre acknowledges the fellowship and the contingency grant received from University Grants Commission, India.

REFERENCES

1. **Brunt, J. V.** 1986. More to hyaluronic acid than meets the eye. Biotechnology **4:**780–782.
2. **Charles, M.** 1978. Technical aspects of the rheological properties of microbial cultures. Adv. Biochem. Eng. **5:**1–15.
3. **Dea, I. C. M.** 1987. Mixed polysaccharide systems, p. 367–385. *In* S. S. Stivala, V. Crescenzi, and I. C. M. Dea (ed.), Industrial polysaccharides. Gordon and Breach, New York.
4. **Deavin, L., T. R. Jarman, C. J. Lawson, R. C. Righelato, and S. Slocombe.** 1977. The production of alginic acid by *Azotobacter vinelandii* in batch and continuous culture. Am. Chem. Soc. Symp. Ser. **45:**14–26.
5. **Dische, Z.** 1962. Color reactions of hexuronic acids. Methods Carbohydr. Chem. **1:**497–501.
6. **Evans, C. G. T., R. G. Yeo, and D. C. Ellwood.** 1979. Continuous culture studies on the production of extracellular polysaccharides, p. 51–64. *In* R. C. W. Berkely, G. W. Gooday, and D. C. Ellwood (ed.), Microbial polysaccharides and polysaccharases. Academic Press, London.
7. **Handel, E. V.** 1968. Direct microdetermination of sucrose. Anal. Biochem. **22:**280–283.
8. **Harada, T.** 1977. Production, properties and application of curdlan. Am. Chem. Soc. Symp. Ser. **45:**265–283.
9. **Herbst, H., A. Schumpe, and W. D. Deckwer.** 1992. Xanthan production in stirred tank fermenters: oxygen transfer and scale up. Chem. Eng. Technol. **15:**425–434.
10. **Hestrin, S.** 1949. Reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine and its analytical applications. J. Biol. Chem. **180:**249–261.
11. **Jarman, T. R.** 1979. Bacterial alginate synthesis, p. 35–45. *In* R. C. W. Berkeley, G. W. Gooday, and D. C. Ellwood (ed.), Microbial polysaccharides and polysaccharases. Academic Press, London.
12. **Kang, K. S., G. T. Veeder, P. J. Mirrasoul, T. Kaneko, and I. W. Cottrell.** 1982. Agar like polysaccharide produced by a *Pseudomonas* species: production and basic properties. Appl. Environ. Microbiol. **43:**1086–1091.
13. **Kenne, L., and B. Lindberg.** 1983. Bacterial polysaccharides, p. 278–348. *In* G. O. Aspinall (ed.), The polysaccharides, vol. 2. Academic Press, London.
14. **Lawson, C. J., and I. W. Sutherland.** 1978. Polysaccharides, p. 327–389. *In* J. H. Rose (ed.), Economic microbiology, vol. 2. Academic Press, London.
15. **Lobas, D., S. Schumpe, and W. D. Deckwer.** 1992. The production of gellan exopolysaccharide with *Sphingomonas paucimobilis* E2 (DSM 6314). Appl. Microbiol. Biotechnol. **37:**411–415.
16. **Margaritis, A., and G. W. Pace.** 1985. Microbial polysaccharides, p. 1005–1044. *In* H. W. Blanch, S. Drew, and D. I. C. Wang (ed.), Comprehensive biotechnology, vol. 3. Pergamon Press, Oxford.
17. **Sandford, P. A., and J. Baird.** 1983. Industrial utilization of polysaccharides, p. 412–485. *In* G. O. Aspinall (ed.), The polysaccharides, vol. 2. Academic Press, New York.
18. **Slodki, M. E.** 1987. New bacterial polysaccharides, p. 3–13. *In* S. S. Stivala, V. Crescenzi, and I. C. M. Dea (ed.), Industrial polysaccharides. Gordon and Breach, New York.
19. **Slonekar, J. H., and D. G. Orentas.** 1962. Pyruvic acid, a unique component of an exocellular bacterial polysaccharide. Nature (London) **194:**478–479.
20. **Yuen, S.** 1974. Pullulan and its applications. Process Biochem. **9:**7–9.