0273-2289/95/5501—0045$06.00

# Some Rheological Properties of the Extracellular Polysaccharide Produced by *Volcaniella eurihalina* F2-7

C. CALVO,* M. R. FERRER, F. MARTINEZ-CHECA, V. BEJAR, AND E. QUESADA

*Departamento de Microbiologia, Facultad de Farmacia, Universidad de Granada, Campus Universitario de Cartuja, 18071 Granada, Spain*

Received July 20, 1994; Accepted September 27, 1994

## ABSTRACT

*Volcaniella eurihalina* strain F2-7 synthesizes an exopolysaccharide named V2-7, primarily composed of glucose, mannose, and rhamnose. The effect of chemical and physical factors on solution viscosity was studied. The V2-7 EPS showed pseudoplastic behavior at concentrations over 0.5% w/v. Viscosity decreased with temperature, but the viscosity values were restored after cooling. Freeze–thawing treatment did not affect the rheological properties of its solutions. Addition of inorganic salts produced a diminution of viscosity. However, the most remarkable aspect of V2-7 EPS is the effect of pH on its solutions; it is able to form high viscosity solutions, like a gel, at low pH values even in the presence of inorganic salts. This property, not present in neutral and alkaline solutions, makes it potentially useful for various industrial applications.

**Index Entries:** Exopolysaccharide; rheology; *Volcaniella eurihalina;* halophilic bacteria.

## INTRODUCTION

Microbial extracellular polysaccharides (EPS) may be produced from a wide variety of raw material and represents a kind of high-value polymers with many industrial applications. At present, microbial polysac-

*Author to whom all correspondence and reprint requests should be addressed.

charides have been used as emulsifiers, stabilizers, or as viscosifiers in food, cosmetic, and pharmaceutical industries (*1–3*). Furthermore, it seems that the biggest market is expected to be the oil industry, where the EPS will serve as mobility controller in enhanced tertiary oil-recovery processes (*4–6*).

To a great extent, the commercial use of these products depends on the rheological behavior of their solutions and the way in which this behavior is influenced by such factors as temperature, pH, salt concentration, and shear rate, among others (*2*). In general, it has been demonstrated that each exopolysaccharide in solution has a particular rheological behavior giving rise to a considerably wider diversity than that shown by traditional polymers obtained from plants and marine algae (*7*). Therefore, the great differences found in the properties of bacterial EPS are encouraging researchers to look for a product with the adequate properties for each particular use and therefore of interest to industry, in spite of their cost.

*Volcaniella eurihalina* is a moderately halophilic bacterium isolated from hypersaline soil, which was first described by Quesada et al. in 1990 (*8*). Some strains belonging to this new species produce large mucous colonies in solid media. The preliminary assays carried out in our laboratory showed the ability of this nonpathogenic bacterium to produce an exocellular polymeric substance.

*V. eurihalina* strain F2-7 was selected as the most suitable strain for the study of the production and characterization of its EPS (*9*). *V. eurihalina* F2-7 produced an EPS containing glucose, mannose, and rhamnose. In solutions, it showed a pseudoplastic behavior, and it formed a weak gel at acid pH (*9*). This work was mainly undertaken in order to establish the influence of pH, temperature, and salt concentration on the rheological properties of the polysaccharide produced by *V. eurihalina* strain F2-7.

## MATERIALS AND METHODS

### Microorganism

The organism used in this study was *V. eurihalina* strain F2-7. This microorganism is a moderately halophilic bacterium isolated from hypersaline soil by Quesada et al. (*8*) and with the ability to produce a large amount of exopolysaccharide (*9*). This EPS is designated V2-7.

### Liquid Medium for Inoculum and Growth

MY medium (*10*) containing 1% (w/v) glucose, 0.5% (w/v) yeast extract, 0.3% (w/v) malt extract, and 0.3% (w/v) proteose peptone, supplemented with a balanced mixture of sea salts (*11*) to give a final salt concentration of 7.5% (w/v), was used. All media were adjusted at pH 7.2 with 1*N* NaOH.

## Production, Isolation, and Purification of EPS

As previously described (*9*), 500-mL Erlenmeyer flasks with 30 mL of MY liquid medium were inoculated with 1 mL of a culture of this strain grown for 48 h. Flasks were incubated at 32°C for 8 d without shaking.

Cell-free supernatant fluids were obtained by centrifugation of cultures at 36,000*g* for 60 min in a Sorvall RC-SB refrigerated centrifuge. The EPS was precipitated with 3 vol of cold ethanol. The precipitated polysaccharide was collected by filtration through a gauze and dissolved in distilled water at 60°C. Then, it was purified by ultracentrifugation, dialyzed extensively against distilled water, and finally lyophilized (*9*).

## Rheology

Lypohilized samples of the polymer were dissolved in distilled water or in salt solutions to the desired concentrations. Viscosity measurement of the solutions was determined with a Brookfield viscometer LTV fitted with a small samples adaptor (Brookfield Engineering Laboratories, Stoughton, MA).

The influence of the following parameters on viscosity was tested: polysaccharide concentration, temperature, inorganic salts, and pH. Determinations were made at room temperature (25°C) and at a shear rate of 30 rpm, unless otherwise stated.

### *Polysaccharide Concentration*

Viscosity of polysaccharide aqueous solutions at concentrations of 0.5, 1, 2, 4, and 8% (w/v) and at different shear rates was determined.

### *Thermostability*

The effect of heating-cooling and freeze-thawing treatment on stability of viscosity of EPS solutions was studied, compared to viscosity values reached by nontreated solutions. Heating-cooling treatment was carried out by heating different 2% (w/v) polysaccharide water solutions at temperatures ranging from 50 to 90°C, holding the temperature constant for 20 min, and leaving them to cool until room temperature (25°C). Viscosity measurements were made after cooling.

Freeze-thawing treatment was carried out by keeping the 2% (w/v) EPS solutions at −20°C for times ranging from 1 to 3 wk, and leaving to thaw at room temperature (25°C). Viscosity measurements were made after thawing.

### *Inorganic Salts*

The influence of ionic strength of the medium on viscosity was studied by dissolving the lyophilized polymer to reach a 2% (w/v) concentration in sea salt solutions (*11*), prepared at concentrations ranging from 0.5 to 20% (w/v). For example, the composition of a 7.5% (w/v) solution was the following in g/L: NaCl, 51.3; $MgSO_4 \cdot 7H_2O$, 13.0; $MgCl_2 \cdot 6H_2O$, 9.0; KCl, 1.3; $CaCl_2 \cdot 2H_2O$, 0.2; NaBr, 0.15; and $NaHCO_3$, 0.05. The viscosity in these conditions was determined.

In order to know the effect of each one of the inorganic salts on viscosity, 2% (w/v) EPS water solutions were added with 2.5, 5, 7.5, 10, 15, and 20% (w/v) of different inorganic salts, and the viscosity in these conditions was determined. The following inorganic salts were tested: KCl, NaCl, $MgCl_2$, and $MgSO_4$.

*pH*

The influence of pH on viscosity was studied, using 1% (w/v) water solutions or 1% citric-acid bisodic phosphate buffer solutions (*12*). The range of pH tested varied from 2 to 12 (distilled water solutions) and from 2 to 8 (buffer solutions).

## RESULTS AND DISCUSSION

### Polysaccharide Concentration and Viscosity

As we have reported (*9*), the EPS produced by *V. eurihalina* strain F2-7 has a pseudoplastic non-Newtonian behavior in solution. The relationship between the polysaccharide concentration and the viscosity of the aqueous solutions at different shear rates is shown in Fig. 1A. Viscosity increased with concentration. The effect of shear rate on viscosity of the polymer solutions was markedly decreased in diluted solutions, with the pseudoplasticity disappearing at 0.5% w/v (Fig. 1B).

### Effect of Temperature and Freeze–Thawing on Viscosity

Although it has been previously described that viscosity of V2-7 polysaccharide decreased with increasing temperature (*9*), viscosity values were always restored after heating and further cooling. Figure 2 shows the heating dependence of V2-7 rheology; solutions heated from 50 to 70°C reached higher viscosity values than those reached by nonheat-treated solutions (25°C), and they kept a markedly pseudoplastic behavior.

The viscosity of polysaccharide solutions was quite unstable at room temperature, and even at 4°C. Therefore, in order to have the stock solutions available, the influence of freezing on the viscosity of various solutions was studied. When these solutions were frozen and kept at −20°C for more than 2 wk, the viscosity values obtained at different freeze-storage times showed the same values as those obtained with the solutions before freezing. Therefore, we can say that the polysaccharide produced by *V. eurihalina* strain F2-7 is stable during the freeze–thaw treatment; this property can be of interest in two ways: one of them is related to the storage of their solutions and the other is related to the possibility of being used as viscosifier in products that will be frozen anytime. The resistance of curdlan gels to degradation by freezing and thawing has also been described as having a potential application to frozen food products (*13*).

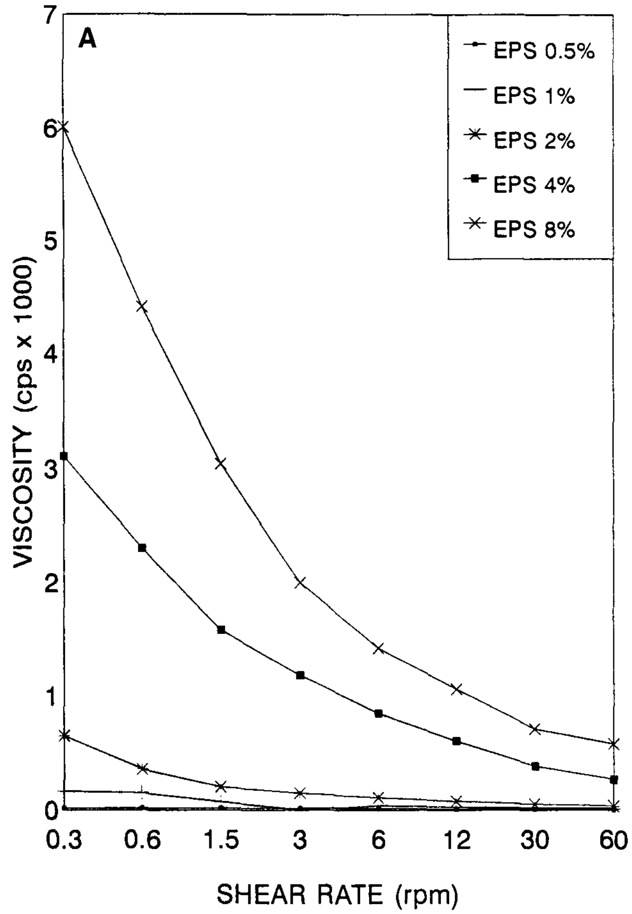


Fig. 1A. Viscosity vs shear rate for various concentrations (w/v) of polymer. Viscosity measurements were carried out at 25°C.

## Influence of Inorganic Salts on the Viscosity

Although many bacterial exopolysaccharides of commercial interest have been evaluated because of their high viscosity, many others may have interest for their behavior with respect to chemical or physical factors (*1,14*). In this way, compatibility with high salt concentration is an important property that can determine the end use of bacterial exopolysaccharides (*1,15*).

As a moderately halophilic bacterium, *V. eurihalina* requires hypersaline conditions for growing and producing exopolysaccharides, and therefore this exopolysaccharide might be specially adapted to salinity. Consequently, we have first evaluated the influence of ionic strength on viscosity by dissolving the polymer in a sea salt solution, the same solution described in the Materials and Methods section, for preparing culture media. Figure 3 shows the relative viscosity values of V2-7 salt solutions relating to water polymer solutions. The viscosity values remained quite

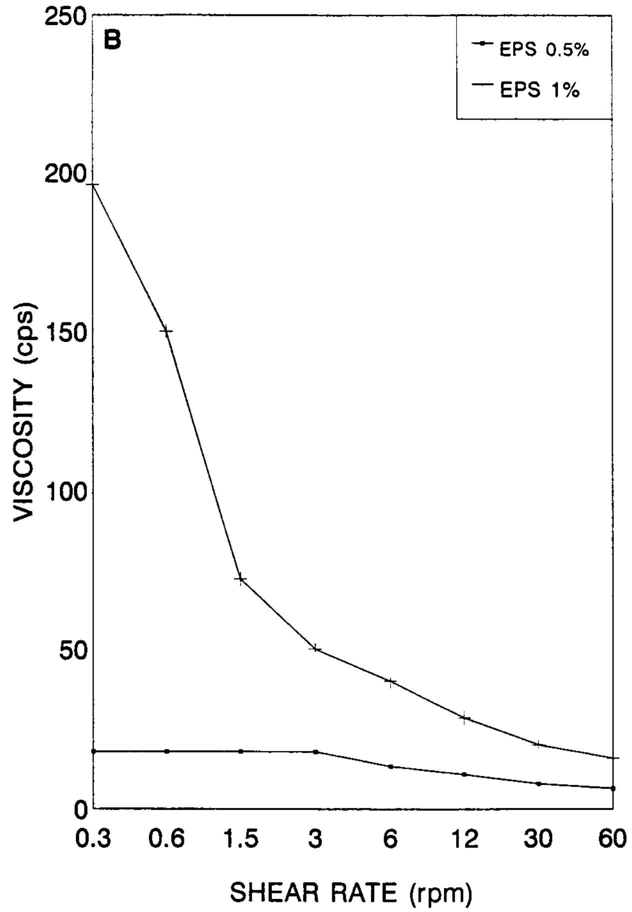


Fig. 1B. Viscosity vs shear rate for 0.5 and 1% (w/v) concentrations of polymer. Viscosity measurements were carried out at 25°C.

constant at lower salt concentrations, with a maximum of viscosity at 2% w/v of salts, but further increase of salt concentration from 2 to 7.5% (w/v) led to a marked decrease of solution viscosity. Nevertheless, an increase of external salt concentration above 7.5% did not give appreciable changes on solution viscosity.

With respect to the effect of each one of the inorganic salts on viscosity, Table 1 shows that the viscosity of the EPS solution decreases when the concentration of any of the inorganic salts assayed increases, although only small differences were found among the viscosity values obtained between 2.5 and 20% w/v of salts. Taking into account the initial diminution of viscosity, we think that V2-7 polysaccharide could have application in high-ionic-strength conditions when high viscosity was not required.

On the other hand, the *in situ* production of xanthan-like polysaccharide in oil-bearing strata has been suggested as a means of aiding tertiary oil recovery (5). *V. eurihalina* V2-7 will grow and produce polysaccharide

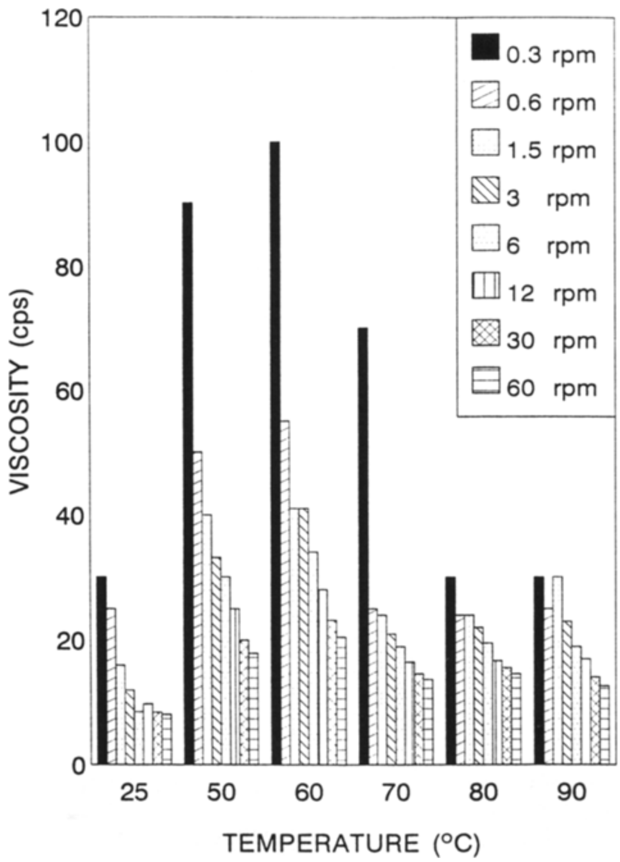


Fig. 2. Effect of temperature treatment on viscosity. Determinations were made at 25°C.

Table 1
Effect of Inorganic Salts on Viscosity

| | Relative value of viscosity, % | | | | | |
|---|---|---|---|---|---|---|
| | Salt Concentration %, w/v | | | | | |
| Inorganic salt | 2.5 | 5 | 7.5 | 10 | 15 | 20 |
| KCl | 32.4 | 26.2 | 24.9 | 24.3 | 24.3 | 24.3 |
| NaCl | 38.5 | 33.7 | 33.2 | 32.7 | 33.4 | 37.7 |
| $MgCl_2$ | 45.6 | 29.6 | 29.6 | 29.6 | 29.9 | 27.6 |
| $MgSO_4$ | 44.5 | 41.8 | 34.6 | 34.2 | 34.2 | 35.2 |

Each inorganic salt was dissolved in 2% (w/v) polysaccharide solution, and the viscosity was measured at 25°C and at 30 rpm.

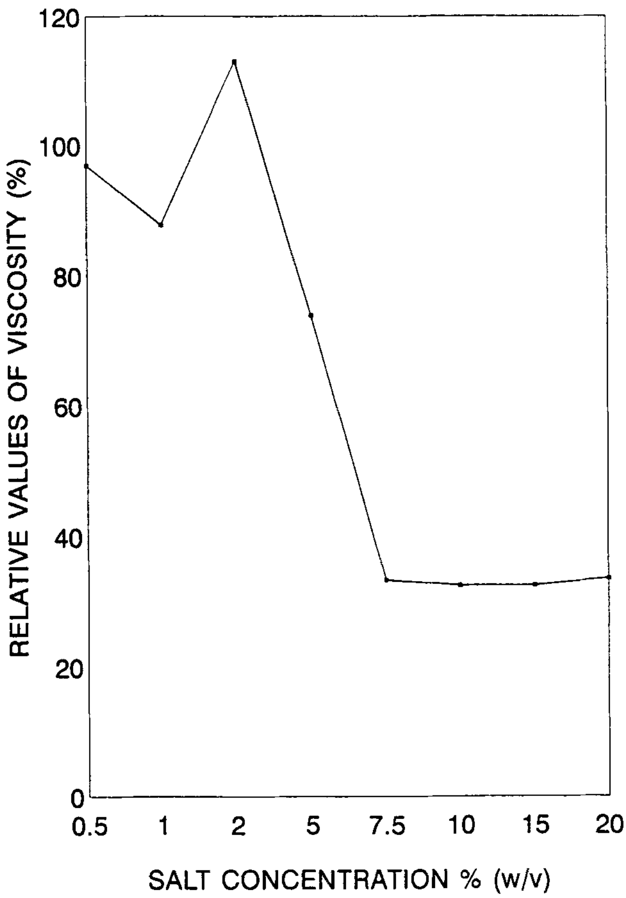


Fig. 3. Effect of salt concentration on viscosity of 2% (w/v) V2-7 polysaccharide in sea salt solution. Measurements were carried out at 25°C and at shear rate of 30 rpm.

in highly saline environments (*9*) as might be encountered in some oil fields, so this ability could make it a candidate for such an application.

## Effect of pH on Viscosity

The most remarkable aspect of V2-7 polysaccharide was the effect of pH on the solution viscosity. Figure 4 shows that viscosity increases when pH value decreases. The ability to form gels, not observed in neutral and alkaline solutions, was found in acidic solutions. Maximum viscosity was produced at pH 2.5 when the polymer was dissolved in distilled water, and at pH 4 in citric buffer solutions. On the other hand, addition of inorganic salts to exopolysaccharide water solutions did not block gel formation (data not shown), and cations, such as magnesium, potassium, or sodium, do not seem to be required for gelation to occur. However, it could be possible that small amounts of any of these cations, present as impurities in the EPS lyophiles, could be involved in gel formation.

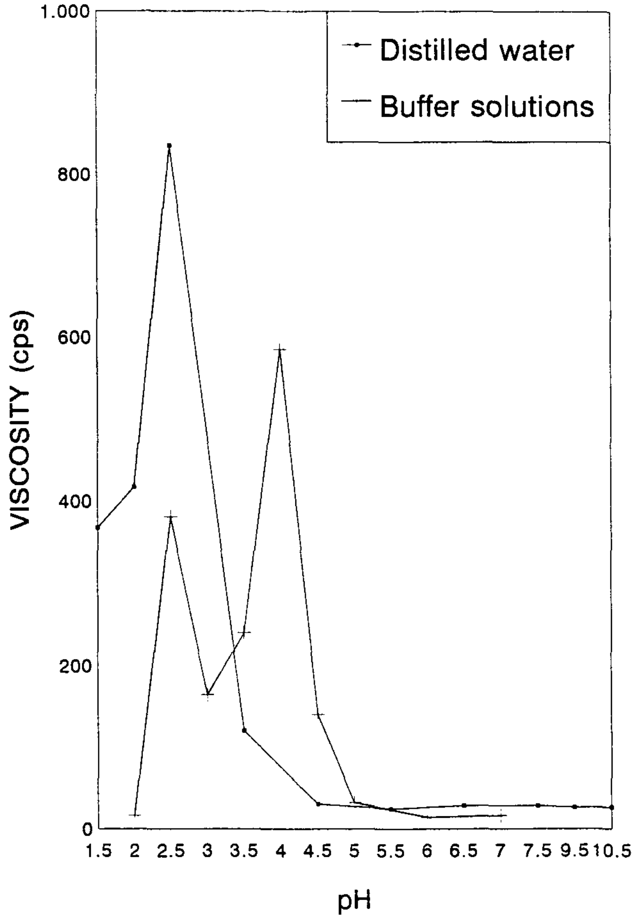


Fig. 4. Effect of pH on viscosity of 1% w/v V2-7 polysaccharide. Measurements were made at 25°C and at shear rate of 1.5 rpm.

Similar to propylene glycol alginate, which does not gel until the pH is below 3 (*1*), V2-7 polysaccharide might be used in food applications, where gel formation is only required when a low pH is reached.

## ACKNOWLEDGMENTS

This work was supported by grant DGICYT, PS88-0105 from Programa Sectorial de Promoción General del Conocimiento.

## REFERENCES

1. Roller, S. and Dea, I. C. M. (1992), *Crit. Rev. Biotechnol.* **12,** 261–277.
2. Kang, K. S., Veeder, G. T., and Cottrell, I. W. (1983), in *Progress in Industrial Microbiology,* vol. 3, Bushell, M. E. ed., Elsevier, Oxford, pp. 231-257.

3. Sutherland, I. W. (1990), in *Biotechnology of Microbial Exopolysaccharide*, vol. 9, Baddiley, J., Carey, N. H., Higgins, I. J., and Pitter, W. G. eds., Cambridge University Press, Cambridge, pp. 141-148.
4. Banat, I. M., Samarah, N., Murad, M., Horne, R., and Banerjee, S. (1991), *World J. Microbiol. Biotechnol.* **7,** 80-88.
5. Knabe, S. (1984), *Oil Gas J.* **82,** 59-60.
6. Behlugil, K., Mehmetoghu, T., and Donmez, S. (1992), *Appl. Microbiol. Biotechnol.* **36,** 833-835.
7. Cesàro, A., Gamini, A., and Navarini, L. (1992), *Polymer* **33,** 4001-4008.
8. Quesada, E., Valderrama, M. J., Béjar, V., Ventosa, A., Gutierrez, M. C., Ruiz-Berraquero, F., and Ramos-Cormenzana, A. (1990), *Int. J. Syst. Bacteriol.* **40,** 261-267.
9. Quesada, E., Béjar, V., and Calvo, C. (1993), *Experientia* **49,** 1037-1041.
10. Moraine, R. A. and Rogovin, P. (1966), *Biotechn. Bioengin.* **VIII,** 511-524.
11. Rodriguez-Valera, F., Ruiz-Berraquero, F., and Ramos-Cormenzana, A. (1981), *Microbiol. Ecol.* **7,** 235-243.
12. McIvaine, T. C. (1921), *J. Biol. Chem.* **49,** 183-187.
13. Phillips, K. R. and Lawford, H. G. (1983), in *Progress in Industrial Microbiology*, vol. 3, Bushell, M. E., ed., Elsevier, Oxford, pp. 201-229.
14. Bodie, E. A., Schwartz, R. D., and Catena, A. (1985), *Appl. Environ. Microbiol.* **50,** 629-633.
15. Anton, J., Meseguer, I., and Rodriguez-Valera, F. (1988), *Appl. Environ. Microbiol.* **54,** 2381-2386.