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Research Paper

Dynamic rheological studies of exopolysaccharides produced by bacteria of *Rhizobium* and *Mesorhizobium* genus

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Abstract

Three different bacteria strains of *Rhizobium* genus were used in the production of exopolysaccharides. EPS R_1 and R_2 were produced from *Rhizobium tropici.* EPS R_3 was produced from a *Mesorhizobium* strain and R_4 from *Rhizobium* sp strain. All the exopolysaccharides were selected for dynamic rheological studies. *Rhizobium* exopolysaccharides demonstrated viscoelastic behavior and strong gelling characteristics. R_3 was the less viscous probably due to a high content of uronic acids (8, 4%) on its structure. Moreover, R_3 exhibited viscous solution behavior at low concentration, and viscoelastic weak gelling at high concentrations. Analysis of temperature influence over exopolysaccharide solutions with viscoelastic behavior showed strong gelling characteristics for R_1, R_2 and R_4 at the range of 5-95ºC (5g/L). In addition, all exopolysaccharides had strong gelling characteristics at 5ºC. These polymers are good candidates to be used in industrial processes, especially involving temperature variations, at least in the range from 5-60°C.

Keywords: Exopolysaccharides, *Rhizobium*, *Mesorhizobium*, Viscoelastic behavior, Gel.

Introduction

Polysaccharides from natural sources are broadly used in many areas of application. Microbial exopolysaccharides (EPS) are considered potential substitutes for those obtained from plants and display physical and chemical properties that are, generally, superior to natural gums [1-2]. The application of these biopolymers in different industrial sectors, such as food and pharmaceutical [3-4] is because they modify the physical properties of the medium, increasing the viscosity of the solutions or creating cohesive intermolecular networks. In food industry, the polysaccharides can be employed as thickeners, stabilizers, emulsifiers, coagulants, film formers, gelling agents, suspending agents and dispersants. Moreover, they also contribute to lower energy intake in dietary products [5-9]. Therefore, understanding the viscous properties of polysaccharide solutions is helpful, for example, to predict their gelation or thickening properties in aqueous system, so that the manufacture, distribution, storage and consumption of products are facilitated [10].

Aranda-Selverio *et. al*. [11] studied extracellular polysaccharides produced by bacteria from *Rhizobium* and *Mesorhizobium* genus and observed that these solutions had non-Newtonian behavior, with pseudoplastic characteristics, suggesting a biotechnological application of these polymers. However, a deeper insight on the biopolymer rheological properties is required, for example to evaluate how

they are affected by temperature. Rheological characterization of exopolysaccharides produced by microorganisms can lead to improvements for predicting desirable functional properties of polysaccharides products.

Materials and Methods

Materials

Three different bacteria strains of *Rhizobium* genus were used in the production of exopolysaccharides. EPS R_1 and R_2 were produced from *Rhizobium tropici* (data are available in the PI0304053-4) [12]. EPS R_3 was produced from a *Mesorhizobium* strain and R_4 from *Rhizobium* sp strain.

General Methods

The exopolysaccharides were individually dialyzed (72 hours in current distilled water) in tubes of 12 kDa of molecular weight cut off, concentrated at low temperature (38 ºC) and lyophilized.

Dynamic rheological measurements

Polysaccharide solutions with concentrations of 2, 5 and 10 g/L were prepared by dispersing the dried hydrocolloids in water at 20 ºC under constant stirring. Rheological measurements using small amplitude oscillatory shear were performed on the samples, using an HAAKE RS-75 Rheostress rheometer with a cone-plate sensor C60/2Tiº, coupled to a circulating HAAKE K-15 bath, and an HAAKE Peltier TC-81 heating system. The experiments were conducted in two steps: (1) deformation sweeps and (2) frequency sweeps.

Deformation sweeps (0.1 and 100 Pa) of the samples were evaluated at 25 ºC and constant frequency of 1 Hz. The experiments were performed in order to determine the maximum deformation attainable by all samples in the linear viscoelastic range. Frequency sweeps of the samples were evaluated at 5 ºC and 25 ºC. The mechanical spectra were characterized by G', G" as a function of frequency (f (Hz)) in the range of 0.01– 10 Hz. G' is the dynamic elastic or storage modulus, related to the material response as a solid. G" is the dynamic viscous or loss modulus, related to the material response as a fluid [13-15]. Temperature sweeps were performed in polysaccharide solutions at 5 g/L. Heating (5 – 95 ºC) and subsequent cooling (95 – 5 ºC), were performed at a rate of 1.5 ºC/min, 1 Hz and 0.6 Pa. Before starting the experiments, the exposed sample edge was covered with a thin layer of low viscosity mineral oil to minimize evaporation losses during measurements. Rheological data were computed from raw oscillatory data using Rheowin Pro Job/Data Manager Analysis software (version 3.20).

Results and Discussion

Dynamic rheological properties of EPS R_1, R_2, R_3 and R_4 at different concentrations

The values of shear stress for frequency sweeps at concentrations of 2 g/L and 10 g/L of the four EPS were 0.2 Pa and 1.5 Pa, respectively. At the concentration of 5 g/L, the shear stress were 0.6 Pa and 0.3 Pa for R_1, R_2, R_4 and R_3, respectively. In these conditions, the polymers displayed a linear viscoelastic behavior, in other words, the applied deformation did not alter the physical structure of the EPS.

The solutions R_1, R_2 and R_4 at 2 g/L concentration exhibited viscoelastic behavior of weak gels (Figure 1). Torres *et. al.* [16] observed similar results in solutions of chitosan polymer at 35 g/L concentration. Additionally, R_1 and R_2 demonstrated gelling behavior, with G' values higher than G'', at 5 g/L concentration. At the same condition, R_4 displayed a viscoelastic behavior of weaker gel, with viscous modulus (G'') higher than elastic modulus, at low frequencies. Solutions of 10 g /L of R_1, R_2 and R_4 presented G' values higher than G''. Independent of the frequency, these EPS had viscoelastic behavior with solid character which is a typical behavior of strong gelling [13].

In the 2 g/L and 5 g/L R_3 solutions was observed the predominance of G'' over G', indicating viscous solution behavior. This, probably, is related to the high content of uronic acid (8,4 %) in this EPS [11]. A similar situation was described by Martínez-Ruvalcaba *et. al.*[17] in 0.1 g/L xanthan solutions. In addition, 10 g/L R_3 solution had viscoelastic behavior of weak gel.

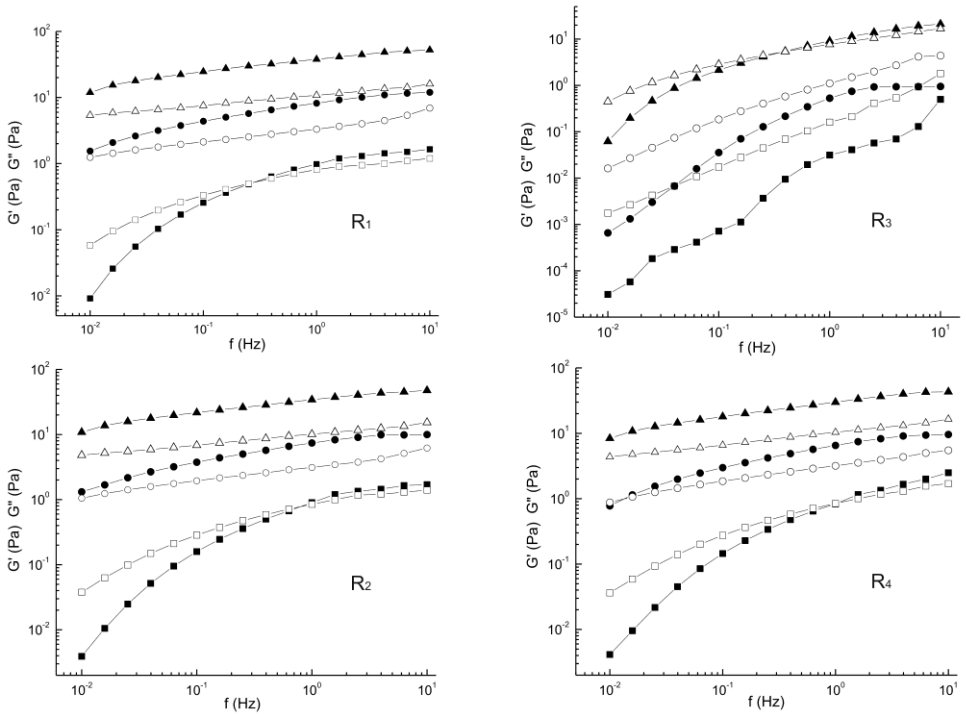

Figure 1: Frequency dependence of storage modulus (G') and loss modulus (G") of exopolysaccharides R_1, R_2, R_3 and R_4 aqueous solutions at 25 °C: 2g/L (—■—), 5 g/L (—●—) and 10 g/L (—▲—). Filled and open symbols represent G' and G", respectively.

The dependence of elastic modulus (G') with the polymer concentration in the frequency of 10^{-1} and 10^{0} Hz is shown in the Table 1. The results demonstrated that G' increases with increasing concentration of the polymers and it can be explained by more junction points as well as the size of junction points among the molecules [17-18]. The data of elastic modulus (G') observed in Table 1 support that R_1 has a stronger gel behavior when compared to R_2 and R_4, while R_3 forms viscous solutions and weak gels.

Table 1: Effect of frequency on storage modulus (G') of exopolysaccharides R_1, R_2, R_3 and R_4, at 25 °C and 5 ºC, cross-over point (Hz)

		G' (Pa)			
		10^{-1} (Hz) * 25 ºC	10^{0} (Hz) * 25 ºC	10^{0} (Hz) * 5 ºC	Cross-over point (Hz) *
R_1	2 g/L	0.25	0.98	---	0.28
	5 g/L	4.37	8.21	9.74	---
	10 g/L	24.60	37.83	---	---
R_2	2g/L	0.15	0.86	---	0.91
	5 g/L	3.5	7.22	7.5	---
	10 g/L	21.72	34.20	---	---
R_3	2 g/L	0.00	0.01	---	---
	5 g/L	0.03	0.52	0.97	---
	10 g/L	2.14	9.08	---	0.36
R_4	2 g/L	0.14	0.83	---	1.31
	5 g/L	2.98	6.52	7.10	0.01
	10 g/L	19.22	32.09	----	---

*Frequency (Hz)

In the cross-over point (also called gelling point) occurs the inversion of G' and G'' values, indicating the viscoelastic behavior of the material. When the cross-over point shows lower values, there is a greater contribution of elastic modulus (G') in the system [19-20]. Data in the Table 1, demonstrate that at low concentrations (2 g/L) the cross-over point was lower in R_1 forming a weak gel. It is also possible to observe that at a high concentration (10 g/L) only R_3 exhibited a cross-over point, demonstrating a weak gel behavior. The fact that R_3 do not have cross-over point at 2 and 5 g/L concentrations demonstrates that the polymer form viscous solutions at low concentrations. Besides that, the viscous modulus (G'') remains above the elastic modulus (G') across the frequency range that was examined (Figure 1). At 5 g/L R_4 solution has lower cross-over point than R_1 and R_2 values (Table 1).

Simsek *et. al.*[21] performed a study with a succinoglycan polymer produced by *Sinorhizobium* bacteria strains. They found different cross-over point values and suggested that this fact could be explained by the presence of charged substitutes in the molecule, like pyruvate and succinate.

The value of elastic modulus (G') at 5 ºC is higher than 25 ºC, at 5 g/L concentration for all EPS (Table 1) demonstrating the formation of stronger gels at low temperatures. Mohammed *et. al.*[22] described a similar behavior for xanthan gum solutions at 5 g/L.

Temperature sweep of EPS R_1, R_2, R_3 and R_4 at 5 g/L

The values of elastic (G') and viscous (G'') modulus of R_1, R_2 and R_4 (Figure 2) do not display large variations in the range of temperature of 5 - 60 ºC. Nearly to 60 ºC (black line), the molecule structure is unstable and loss the gel strength. When the temperatures are higher these molecules become disordered[23]. At low temperatures, the molecules have an organized conformation.

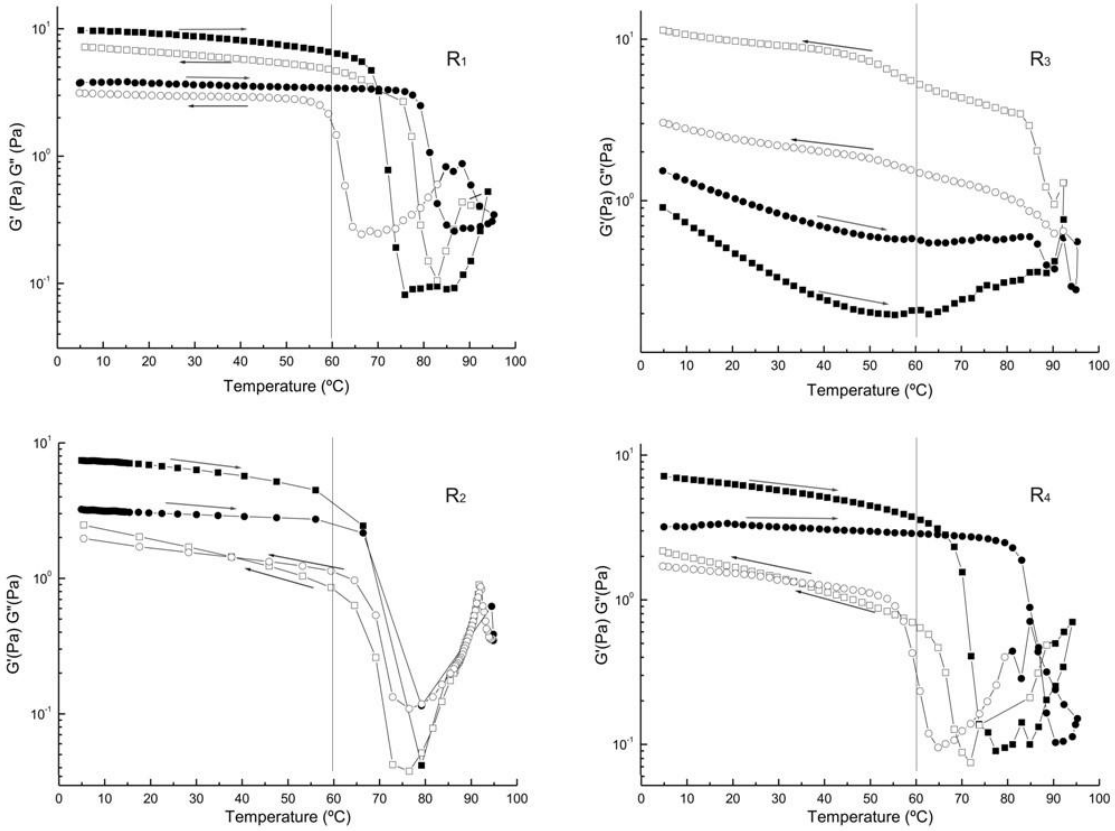

Figure 2: Variation of G' (—■—) and G" (—●—) (1 Hz, 0,6 Pa) during temperature sweeps for 5 g/L of exopolysaccharides R_1, R_2, R_3 and R_4 aqueous solutions. Filled symbols represent heating from 5 to 95 ºC and open symbols represent cooling from 95 to 5 ºC

The 5 g/L R_3 solution showed a weak gel behavior during the heating (5 - 95 ºC), with G'' higher than G'. At the cooling phase (95 - 5 ºC), it formed a strong gel with G' higher than G''. García-Ochoa *et*

al.[23] observed that the viscosity of xanthan gum decreased when the temperature increasing until 40 ºC. In the range of 40 - 60 ºC the viscosity increased. For temperatures higher than 60 ºC, the viscosity decreased. The strong temperature dependence is attributed to a conformational transition of xanthan chains from helical at low temperatures to random coil at high temperatures. These variations in the viscosity probably are due to charges interactions in the xanthan molecule[24]. A similar condition may occur in R_3 considering the high density of negative charges.

Temperature sweep of R_4 solution (Figure 2) showed that the polysaccharide formed a thermo resistant gel during the heating phase, with G' higher than G''. During the cooling phase, until 30 ºC, G'' is superior to G'. In this temperature, an inversion of values was observed, with formation of a strong gel at low temperatures.

Conclusion

The results of non-destructive oscillatory measurements demonstrated that the EPS produced by *Rhizobium* and *Mesorhizobium* had gelling viscoelastic behavior. R_1 formed the strongest gel, followed by R_2 and R_4. However R_3 formed a viscous solution and presented a weak gel behavior at 10 g/L concentration. All EPS demonstrated a strong gelling behavior at low temperatures. These polymers are good candidates to be used in industrial processes, especially involving temperature variations, at least in the range from 5 - 60 ºC.

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