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MODULATION OF *Porphyridium aerugineum* POLYSACCHARIDE RHEOLOGY BY AQUEOUS EXTRACT OF A GARDEN SOIL.

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ABSTRACT

A stock (0.5% w/v) aqueous solution of the polysaccharide of the microalga *Porphyridium aerugineum* was further diluted using (i) deionized water and (ii) an aqueous (0.2% w/v) solution of a new, garden soil extract. The viscosity of the resultant solution was higher by about 23% (5 samples) where the soil extract was used. The viscosity of the soil extract alone was very low i.e., 0.62 ± 0.074 centipoise for a 0.2% w/v solution. It is indicative that the soil extract may be acting synergistically to change the conformation of the algal polysaccharide chain molecules, resulting in the observed higher viscosity. The soil extract also enhanced thermostability of low aqueous solutions of the *P. aerugineum* polysaccharide. A comparison of the Infra Red (IR) spectra of the new soil extract and the *P. aerugineum* polysaccharide shows that the two substances are essentially different. With this new soil extract the mechanism of synergism in the rheology of the *P. aerugineum* polysaccharide can be further elucidated and exploited for commercial, food and industrial applications.

KEY WORDS: *P. aerugineum*, polysaccharide, rheology, soil extract

INTRODUCTION

Porphyridium aerugineum is a red microalga and its polysaccharide has been characterized with high viscosity resulting from low concentrations in aqueous solutions. The polysaccharide is sulfated, and has protein moieties attached to it (Percival and Foyle, 1979, Geresh and Arad, 1991). The rheological properties of *P. aerugineum* polysaccharide make it attractive for use as a viscofier, suspender or thickener in food and industrial applications (Geresh and Arad, 1991). There are still other desirable properties that can be realized from this and other biopolymers when they are modified or mixed with other substances. Such desirable properties include high viscosity and thermostability at low concentrations. This is not always readily achievable, especially since many polysaccharide solutions show changes in rheology on heating (Feuerstein and Franz, 1995). One way these properties can be realized is by polymer interaction leading to synergism (Geresh and Arad, 1991). Rheological synergism is also considered of great importance in the phenomenon of mucoadhesion or bioadhesion (Rossi et al., 1995), where certain polymers have been shown to interact with cellular mucin leading to bonding.

The purpose of this study was to carry out a preliminary investigation on the effect of a new garden soil extract on the rheology of *P. aerugineum* polysaccharide in aqueous solutions. This will enhance the application of aqueous solutions of the polysaccharide in food and industrial ventures and basic research.

METHODS

In an attempt to discover carbohydrolases or polysaccharidases for algal polysaccharides, a total of five soil samples, were collected from different spots at the Institutes for Applied Research, Ben Gurion University of the Negev, Beersheva and incubated with aqueous solutions of the polysaccharide of *Porphyridium aerugineum*. The polysaccharide solutions were first sterilized by autoclaving to eliminate enzymatic activity before being incubated with the soil materials. When the solutions of *P. aerugineum* polysaccharide in deionised water (dH_2O) were sterilized by autoclaving at 11psi for 35 min there was a drop in viscosity of about 20-32%. The resultant solution was mixed with the soil samples and the mixture was monitored for cleavage (carbohydrolase ability). Preliminary investigations indicated that one of the soil samples, mainly of peat material, was able to restore up to 80% drop in viscosity of the *P. aerugineum* polysaccharide solution in deionised water. This phenomenon was noted. Normally a decrease in viscosity was the desired effect, which would be indicative of the presence of carbohydrolases. To investigate this

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phenomenon, the peat soil material was extracted for 48-72 h in deionised water, and first filtered, through whatman #4, and then #41. The filtrate was first dried by rotavaporation, and finally over $CaCl_2$ in a dessicator. The resulting material was soluble in deionised water with an amber color. Next, an aqueous solution of *P. aerugineum* polysaccharide was further diluted with deionised water on the one hand, and on the other hand, with an aqueous solution of the new soil extract (SE). The experiment was performed with five separate but equal amounts of both the soil extract and the polysaccharide (Table 1). The pH of the aqueous solutions was measured with a digital pH meter Model 8521 (HANNI Instruments, Italy), and the viscosity was measured with a Brookfield viscometer (Brookfield, LTV), shear rate at 30 S^{-1}. Both the pH and the viscosity measurements were done at ambient temperature (22-24 °C). In order to establish the chemical nature of the new soil extract, the garden soil was extracted for 24 h using absolute methanol. The filtrate, obtained after filtering through whatman #4 and then #41, was rotavaporated and further dried over $CaCl_2$. About 2-4mg of the dried material was pelleted using KBr, and an Infra Red (IR) spectrum was produced from a thin film of the pellet.

RESULTS AND DISCUSSION

Table 1 is a summary of different treatments of the *P. aerugineum* polysaccharide and the soil extract with the respective outcome. There was no change in viscosity and pH of aqueous solutions of the soil extract after autoclaving. There was a 24% drop in the viscosity of both the treated polysaccharide (PSS+SE) and the untreated polysaccharide (PSS in dH2O) after autoclaving. The effect of heating on the rheology of *P. aerugineum* polysaccharide is in line with other reports (Feuerstein and Franz, 1995). The viscosity of the treated polysaccharide was higher than the untreated one, both before, and after autoclaving. This indicates that the soil extract had an enhancing effect on the viscosity of the polysaccharide. The viscosity of the untreated samples that were not autoclaved remained stable; similarly the viscosity of samples that were treated with the soil extract but not autoclaved remained stable for long periods of time (ca. 3 weeks). This indicates that the effect of the soil extract on the biopolymer is stable. All samples were handled under ordinary lighting conditions. The Infra Red (IR) spectra of the soil extract and the *P. aerugineum* polysaccharide are given in Fig 1. The OH stretch is very broad 3000 – 3500 cm^{-1} for the *P. aerugineum* polysaccharide (Fig 1B). The stretch is more compact for the soil

Table 1. Different treatments of the *P. aerugineum* polysaccharide and the outcome.

Treatment	SE in dH2O (0.2%)	SE+PSS (2:1 v/v)	PSSin dH2O (0.2%)	n (%)
Not autoclaved				
Viscosity	0.62(0.074)	37.8(1.2)	31.1(1.1)	23
pH	6.9(0.027)	7.5(0.11)	7.97(0.06)	
Aautoclaved*				
Viscosity	0.61	28.7(1.4)	23.6(1.4)	21
pH	6.8	7.8(0.046)	8.1(0.053)	
▼n (%)	0	24(2.8)	24(1.9)	

*Autoclaving was done at 11psi for 35 min, cooled for 24 h.
SE – is soil extract; PSS – is polysaccharide.
Standard deviation (sd) values are in bracket and are for five samples (n=5).
▲n is increase in viscosity as a result of the treatment of PSS with SE i.e., going left to right.
▼n is drop in viscosity resulting from autoclaving i.e., going top to bottom. Note that there is a drop in both the treated and the untreated PSS after autoclaving, but the resultant viscosity of the treated sample was still higher than the untreated.

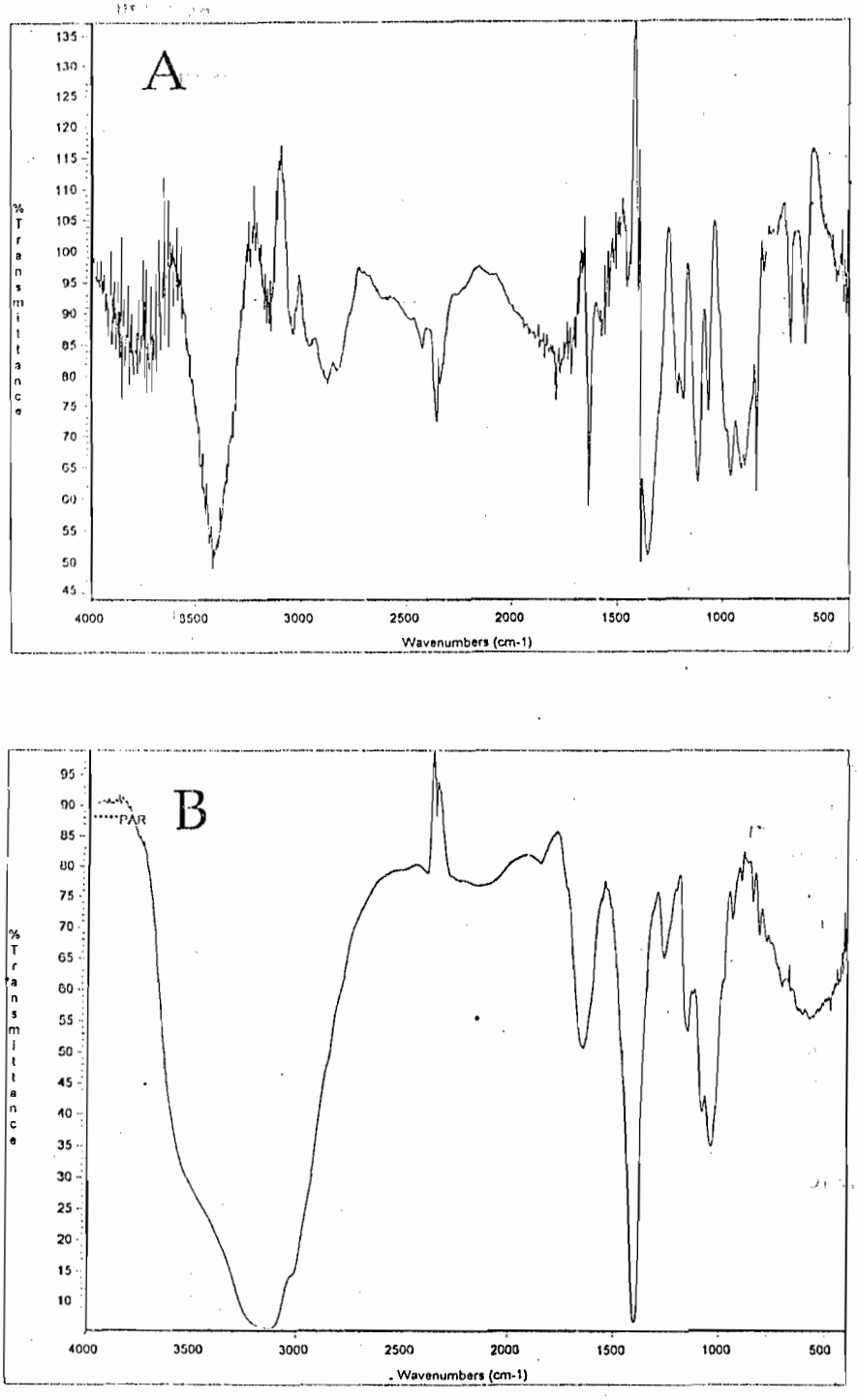

Figure 1. Infra Red (IR) spectra of the new soil extract (A) and the *P. aerugineum* polysaccharide (B).

extract (Fig 1A). This indicates that the soil extract is more sparsely hydroxylated. The CH stretch is very distinct at 2600 – 2800cm^{-1} in the soil extract (Fig 1A) compared to the polysaccharide (Fig 1B). The sharp signal at 1650cm^{-1} (Fig 1A) is indicative of carboxyl (COO) stretch, it is more broad in the polysaccharide (Fig 1B). The stretch between 1300 and 1400cm^{-1} is indicative of sulfur groups, and is distinct in both materials. It is clear, however, that the two substances are essentially different. On the basis of the foregoing the new soil extract can be putatively identified as a material of sulfated humic substances. It is possibly a combination of humic and tannic acid complexes since it was derived from peat-like material. It has low viscosity compared to the polysaccharide (Table 1). This is the first report of a soil extract having this kind of effect on the rheological properties of the *P. aerugineum* biopolymer. Chitin substances such as chitosans are common in soil but aqueous solutions of chitosan usually have much higher viscosity than what was observed for the garden soil extract. The effect of the new soil

extract is thought to be synergistic. Viscosity changes are generally attributable to changes in the conformational structure of polymer chain molecules. We thus infer that there could be a positive synergistic interaction between the soil extract and the *P. aerugineum* polysaccharide, which results in a change in the conformation of the polysaccharide chain molecules leading to changes in rheology. This may be as a result of physical chain entanglements and non covalent bonds between the soil extract and the *P. aerugineum* polysaccharide. This in turn leads to the observed increase in viscosity. Rheological synergism has been reported between *P. aerugineum* polysaccharide and locust bean gum (Geresh and Arad, 1991). It is also observed that the new soil extract preserved, to an extent, the native state of the *P. aerugineum* polysaccharide during thermal treatment. This observation is based on the fact that the viscosity of the polysaccharide treated with the soil extract was higher than the untreated one before and after autoclaving. The thermal treatment did not reverse the effect of the soil extract on the *P. aerugineum* polysaccharide. Rheological synergism in the *P. aerugineum* polysaccharide can be exploited in such areas as formulations, thickeners, and in flow control.

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