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Research article

Yield and rheological properties of exopolysaccharide from a local isolate: *Xanthomonas axonopodis* pv. *vesicatoria*

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ABSTRACT

Background: The aim of the present study was to evaluate gum productivity of a local strain, *Xanthomonas axonopodis* pv. *vesicatoria*, isolated from pepper plant, and its rheological behavior for the first time compared to the standard strain, *Xanthomonas campestris* DSM 19000 (NRRL B-1459). The influence of operational conditions (agitation rate and inoculum volume) on gum production and rheological properties of gums from the *Xanthomonas* strains were investigated.
Results: The isolated strain of *Xanthomonas* showed similar xanthan yield compared to the standard strain. Furthermore, this study clearly confirmed that gum yield depended on bacterial strain, agitation rate, and inoculum size. The most suitable conditions for the gum production in an orbital shaker in terms of agitation rate and inoculum size were 180 rpm and 5%, respectively, resulting in an average production of 10.96 and 11.19 g/L for *X. axonopodis* pv.*vesicatoria* and *X. campestris* DSM 19000, respectively. Regarding the rheological properties, Ostwald-de-Waele and power law models were used to describe flow and oscillatory behavior of the gum solutions, respectively. Consistency of the novel gum solution remarkably was much higher than the commercial xanthan gum solution. Flow and oscillatory behavior and their temperature ramps showed that weak gel-like structure could be obtained with less gum concentrations when the novel gum was used.
Conclusion: Therefore, yield and technological properties of the aqueous solutions of the exopolysaccharide synthesized by *X. axonopodis* pv. *vesicatoria* were observed to be more suitable for industrial production.

1. Introduction

It has been reported that natural polymers such as polysaccharides have been recently the focus of interest because of their outstanding properties including biocompatibility, biodegradability, non-toxicity, and renewability [1]. Xanthan gum is an extracellular heteropolysaccharide that is produced biotechnologically by *Xanthomonas* spp. [2]. This gum is authorized by the U.S. Food and Drug Administration for application as food additives without any restrictions [3].

Xanthan gum when dispersed in water quickly produces a viscous, stable solution, even at low concentrations. Because of gum pseudoplasticity, its solution in water is a suitable stabilizer, thickener, and suspending agent in many foods [4]. Today xanthan gum is commercially the most important microbial polysaccharide. Worldwide consumption of xanthan in 2014 was estimated between 150,000 and 160,000 metric tons [5].

In this regard, developing a local strain of *Xanthomonas* for xanthan production is of importance. Composition, viscosity, and yield of xanthan varies depending on the *Xanthomonas* strain used in the production. Therefore, local isolates that can be used in the production of xanthan with good quality attributes should be investigated.

Commercial interest in the xanthan gum is due to its rheological properties [5]. Therefore, rheological properties and exopolysaccharide stability properties of these isolates should be investigated prior to their introduction to commercial use. The species, pathovar, and strain infuence of *Xanthomonas* on the rheological behavior of xanthan produced has been investigated [6,7,8,9]. Most of the previous research on microbial xanthan production has focused on the type of carbon source [10,11,12] and optimization of operating conditions [13,14].

Production parameters during fermentation process and the strains used in the production have an effect on the yield and the properties of xanthan gum [15,16].

Therefore, the evaluation of these parameters for the optimization of the production of xanthan is also important. Potential use of these strains can be evaluated by essentially determining the optimal production parameters.

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Therefore, in this study, the properties of wild-type strain of *Xanthomonas* isolated from pepper including gum rheology and xanthan gum production at different conditions of agitation rate and inoculum volume were investigated. The obtained results were compared with those of the *X. campestris* DSM 19000 standard strain to evaluate the yield and the quality parameters of the gum produced using the wild-type strain of *Xanthomonas*.

2. Materials and methods

2.1. Isolation and Identification of Microorganisms

Xanthomonas axonopodis pv. *vesicatoria* was isolated from pepper (*Capsicum annuum* L.) in Turkey. The identification process was performed by conducting morphological, biochemical, and physiological tests, including KOH solubility for Gram reactions, catalase reaction, oxidative/fermentative metabolism, and hypersensitivity to tobacco leaves. Identification of the strain was confirmed by fatty acid methyl ester (FAME) analysis [17,18].

Xanthomonas campestris DSM 19000 (NRRL B-1459), which is the standard bacterium, was obtained from the Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures (Germany).

2.2. Culture media

A) The organisms were maintained in Yeast Malt (YM) agar containing (g/L): 3.0 yeast extract, 3.0 malt extract, 5.0 peptone, 10.0 glucose, 20.0 agar, and distilled water (pH 7.2) [19]. To verify some morphological characteristics, the strains were transferred every 14 days and stored at $\pm 4^oC$.

B) Cell production was carried out in two stages. In the first stage, a pre-inoculum was prepared using YM agar and incubated at 28 ± 2^oC for 24-48 h until the optical density value at 560 nm reached 3-4 ($OD_{560} = 3\text{-}4$). The inoculum was inoculated in 50 mL YM broth and incubated at 28 ± 2^oC and 180 rpm. Then, 2 mL aliquot of the pre-inoculum was taken aseptically to an Erlenmeyer flask containing 100 mL of YM broth and incubated again at 28 ± 2^oC and 180 rpm. The cells were produced using a pre-inoculum containing up to about 10^8 cfu.mL^{-1}.

C) The xanthan gum production medium comprised 40.0 (g/L) glucose, 2.1 (g/L) citric acid, 2.866 (g/L) KH_2PO_4, 0.507 (g/L) $MgCl_2$, 0.089 (g/L) Na_2SO_4, 0.006 (g/L) H_3BO_3, 0.006 (g/L) ZnO, 0.020 (g/L) $FeCl_3 \cdot 6H_2O$, and 0.020 (g/L) $CaCO_3$. The carbon source used for the fermentation studies was glucose [20].

2.3. Xanthan gum production

Yield and viscosity values of xanthan vary depending on microbial strains, colonial variation, media, and the parameters of the fermentation process to obtain the biopolymer [7]. Two process conditions, agitation rate and inoculum volume, were assesed in this study. Xanthan gum was produced in a 1000-mL Erlenmayer flasks containing 500 mL medium. It has been reported that the optimum temperature, fermentation period, and initial pH parameters were 28^{o}C, 72 h, and pH 7.2, respectively [21]. In accordance with the results reported in the previous studies, the system temperature was maintained at 28^{o}C using a temperature-controlled orbital shaker incubator. This procedure was essential as the substrate consumption reactions were exothermic and therefore the temperature of the medium tended to rise. The initial pH of the fermentation medium was 7.2; however, constant pH control was not possible in the shaker. The agitation rate (180-300 rpm) and inoculum size (5% and 10%) levels were studied, and the productivity of the two microorganisms by fermentation was compared in the variable fermentation conditions. All experiments were performed in triplicate. The medium used for fermentation was centrifuged for 30 min for cell separation (SIGMA 2-16KL) at 4^{o}C and 10,000 rpm. Isopropanol (Merck) was added to the supernatant at 1:3 ratio (v/v) for the recovery of the biopolymer. The obtained polymer was dried at 50^{o}C until constant weight. Then, the dried polymer was ground in a disk mill until the granule size reached 0.5 μm. The evaluation of the biopolymers of each strain at different conditions was performed by weighing the the dry product per liter of fermented broth. The average values were determined in g/L.

2.4. Rheological behaviors

The rheological behaviors were determined using three concentrations that are frequently used in food systems (0.5%, 1%, and 2%). Samples were prepared by dissolving the desired amount of dry sample in deionized water with a magnetic stirrer at 40°C. Prepared samples were tempered for 24 h at room temperature before conducting any experiment. Reproducibility of the data was checked by repeating experiments between 3 and 5 times with new samples. Rheological analyses were conducted by suitable models to quantify the properties of xanthan gums.

2.4.1. Steady shear measurements

A controlled stress Discovery Hybrid Rheometer-2 (TA Instruments New Castle, DE, USA) fitted with a parallel-plate geometry (stainless

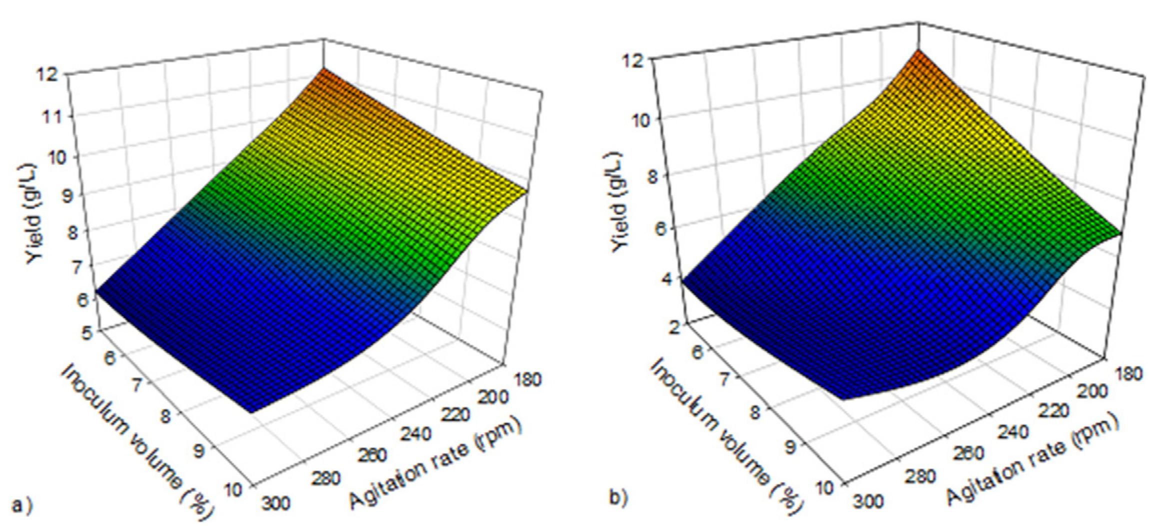

Fig. 1. (a) Effects of inoculum volume and agitation rate on the production of xanthan for *X. campestris* DSM 19000 (g/L) (b) Effects of inoculum volume and agitation rate on the production of xanthan for *X. axonopodis* pv. *vesicatoria* (g/L).

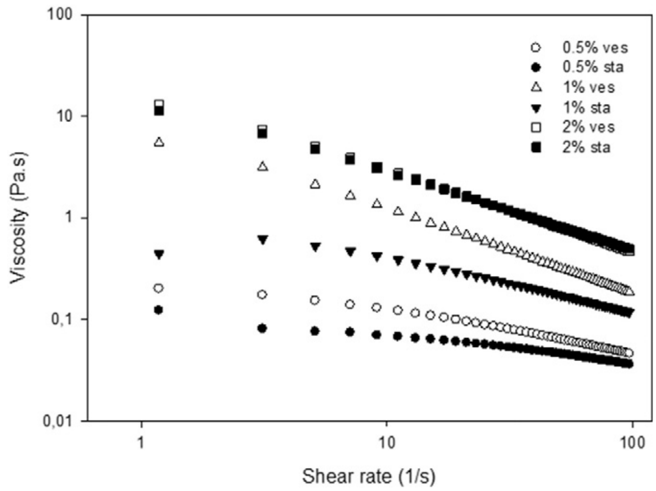

Fig. 2. Viscosity change of different gum solutions obtained from *X. axonopodis* pv. *vesicatoria* and *X. campestris* DSM 19000 against shear rate.

steel, 40 mm diameter, 1000 μm gap) was used for the determination of rheological properties. The shear rate range adopted for xanthan solutions at 0.5, 1, and 2 wt% was 1-100 s^{-1}. Shear rate, shear stress, normal force, torque, and apparent viscosity data were collected during the trials. To determine the flow behaviors of the samples, the Ostwald-de-Waele model was used as follows:

$$\sigma = K(\gamma)^n \quad \text{[Equation 1]}$$

where σ is the shear stress (Pa), K is the consistency coefficient (Pa.s^n), γ is the shear rate (s^{-1}), and n is the flow behavior index (dimensionless).

2.4.2. Dynamic Rheological Measurements

Dynamic oscillatory shear rheometer Discovery Hybrid Rheometer-2 (TA Instruments New Castle, DE, USA) was used to conduct stress sweep and frequency sweep tests for all gum solution. Stress sweep test was used for the determination of linear viscoelastic region. Frequency sweep test was performed at 0.6 Pa over a frequency range of 0.05-100 rad/s. The following power law was used for the modeling of the elastic or storage modulus (G') and the viscous or loss modulus (G"):

$$G' = K'(\omega')^{n'} \quad \text{[Equation 2]}$$

$$G'' = K''(\omega'')^{n''} \quad \text{[Equation 3]}$$

where K', ω', and n' are intercepts, angular frequency, and elastic behavior index, respectively, and K", ω", and n" are viscous counterparts.

2.4.3. Effect of Temperature on the Rheological Parameters

The effect of temperature on viscosity of the gum solutions was also investigated and modeled by Arrhenius equation [22].

$$A = A_0 \exp(E_a/RT) \quad \text{[Equation 4]}$$

where A is the parameter (Pa.s), A_0 is the constant of Arrhenius equation(Pa.s), E_a is the activation energy (kJ/mol), R is gas constant(8,314*10^{-3} kJ/molK), and T is temperature (K).

2.5. Statistical Analysis

The results were statistically analyzed using Minitab for Windows Release 14®. The Duncan's multiple range test was used for the calculation of standard errors.

3. Results and Discussion

3.1. Xanthan yield

Many studies have reported that the strain used in the production of Xanthan had an effect on the xanthan yield and its properties [23,24]. The effects of the parameters for *X. axonopodis* pv. *vesicatoria* and *X. campestris* DSM 19000 in terms of xanthan gum production are presented in Fig. 1. Both inoculum volume and agitation rate were shown to be important factors for xanthan production.

The highest xanthan gum yield values were determined at 180 rpm agitation rate and 5% inoculum volume in broth for both *X. axonopodis* pv. *vesicatoria* and *X. campestris* DSM 19000 to be 10.96 and 11.19 gL^{-1}, respectively. Generally, the isolate of *X. axonopodis* pv. *vesicatoria* presented remarkable and similar xanthan gum yields compared to standard strain in the all experiments.

Previous studies [6,7,25,26,27,28] have reported that the strain used had an effect on the production. The results obtained in the present study confirmed those results. It was concluded that the first step in studies on xanthan production with the highest yield should be the selection of strain.

Regarding the effect of inoculum volume, as can be seen from Fig. 1, higher yields were obtained at 5% inoculum in all agitation rates except 300 rpm. The inoculum volume of 10% facilitated better production of biomass rather than the byproduct xanthan. In particular, for *X. axonopodis* pv. *vesicatoria*, increasing inoculum volume in the medium stimulated the xanthan production dramatically and nearly halved gum production from 10% to 5%. These results showed that the increase in cell concentration had no effect on the increase in xanthan production. Ben Salah et al. [29] reported that the optimum inoculum size for maximum xanthan production using *X. campestris* was 5%. The results obtained in the present study were in line with those obtained by the researchers. Higher amounts of inoculum possibly had no positive effect on the yield as the nutrients and the space for them was not sufficient for active growth. The size of the inoculum can change depending on the strain type. Fernandes-Silva et al. [30], in their study, produced xanthan using cheese whey as substrate for fermentation. The researchers adopted 20% (v/v) 24-h inoculum for production.

As seen in Fig. 1, agitation, in general, had a significant effect on xanthan production because the xanthan yield increased as the agitation values decreased, except at 300 rpm. However, some researchers reported that higher stirrer speed is necessary for xanthan production by *X. campestris* [31], *X. campestris* ATCC 1395

Table 1
Effect of xanthan gum concentration on activation energies, Ostwald-de-Waele parameters, and apparent viscosity of xanthan gum solutions obtained from different isolates at 20°C.

Xanthan gum conc. (%)	Strain	*K* (Pa s^n)	*n* (-)	R^2	*A* (Pa s^n)	Activation energy (kJ/mol)	R^2
0.5	*X. axonopodis* pv. *vesicatoria*	0.375a	0.546b	0.99	9.16*10^{-6}a	23.5a	0. 98
	X. campestris DSM 19000	0.154b	0.688a	0.99	1.98*10^{-6}b	25.43a	0.99
1	*X. axonopodis* pv. *vesicatoria*	8.098a	0.178b	0.98	0.07a	6.78b	0.96
	X. campestris DSM 19000	1.445b	0.457a	0.98	7.4*10^{-5}b	21.25a	0.98
2	*X. axonopodis* pv. *vesicatoria*	18.619a	0.196b	0.99	0.07a	9.07a	0.97
	X. campestris DSM 19000	16.295b	0.236a	0.99	0.046b	10.12a	0.97

K: consistency index; n: flow behavior index; A: constant determined from the Arrhenius relationship ; R^2: determination coefficient. Different lowercase letters show differences between the columns ($P < 0.05$).

[32], *X. campestris* ATCC 33913 [33], *X. campestris* pv. *mangiferaeindicae* IBSBF 1230 [19], *X. arboricola* pv *pruni* 106 [34], and *X. campestris* PTCC 1473 [35]. Nevertheless, our results were in agreement with that of Ben Salah et al. [29] who evaluated xanthan production at distinct stirrer speeds (50, 180, and 250 rpm) and obtained highest levels of xanthan gum at an agitation speed of 180 rpm. Ben Salah et al. [29] have reported that lower xanthan gum values were associated with the bacterial fragmentation due to mechanical shearing. According to the results, it can be speculated that depending on the operational conditions, there is an optimum mixing rate that does not cause bacterial damage and at the same time does not limit mass transfer. Generally, microorganism investigated in this study did not resist high agitation probably because of vulnerable cell structure against hydrodynamic stresses. These results confirm that the yield depended on operational conditions and bacterial strain.

3.2. Rheological properties of xanthan gums

3.2.1. Steady shear properties

The gums produced by both microorganisms were also evaluated rheologically. Fig. 2 shows that the samples had a pseudoplastic behavior, resulting in an apparent decrease in viscosity the increase in shear rate. Generally, solutions of exopolysaccharides obtained from microorganisms show such behavior [19,31]. At all gum concentrations of the solutions, gum from the local strain (*X. axonopodis* pv. *vesicatoria*) remarkably showed higher viscosity than the commercial xanthan gum obtained from the standard strain (*X. campestris* DSM 19000). Ostwald-de-Waele model was used to fit experimental viscosity versus shear rate data to compare the non-Newtonian behavior of the solutions and can be seen in Table 1. R^2 values were higher than 0.98, indicating good fitting of the model. As could be seen from K (consistency index) values, gum from *X. axonopodis* pv. *vesicatoria* formed solutions of higher viscosity at all concentrations. However, n (flow behavior index) was lower than that of the standard strain, indicating low stability against shear. Therefore, when the shear rate increased, more pronounced decrease was obtained in the gum solution from *X. axonopodis* pv. *vesicatoria*. In one of the study that investigated 150 wild strains of *Xanthomonas, Xanthomonas campestris* ICa-125 strain isolated from cabbage showed lower viscosity than the standard strain [36].

Concerning the effect of temperature on the viscosity values of gum solutions at 10 s^{-1} shear rate, generally, a decrease was observed as expected in Fig. 3. Increasing the gum concentration of the solutions led to a sharp decrease in the viscosity values with increase in temperature. The Arrhenius model was used to compare viscosity change of solutions with respect to temperature. R^2 values were between 0.96 and 0.99 (Table 1). It was clearly seen that activation energies changed between 6 and 25 kJ/mol. Moreover, a concentration increase resulted in decrease in activation energies, as expected for xanthan gum solutions [37]. Similar temperature stability of the gum solutions, except for 1% gum concentrations, was observed because of similar activation energies for both gums.

3.2.2. Dynamic rheological properties

Regarding the viscoelastic properties of gum solutions, Fig. 4 shows the oscillatory frequency sweep tests in linear viscoelastic regions of the studied gums. Storage (G') and loss (G") modulus values increased with gum concentrations because of increase in the interaction between biopolymer molecules. These values also increased with angular frequency, showing dominance of elastic response at higher frequencies. The solutions of both types of xanthan gums demonstrated weak-gel behavior as both G′ and G″ values and the difference between them increased with polymer concentration. However, crossover frequency was observed at 1% and 0.5% concentration of gum from *X. campestris* DSM 19000 and 0.5% of *X. axonopodis* pv. *vesicatoria,* indicating the occurrence of macromolecular entanglements that were

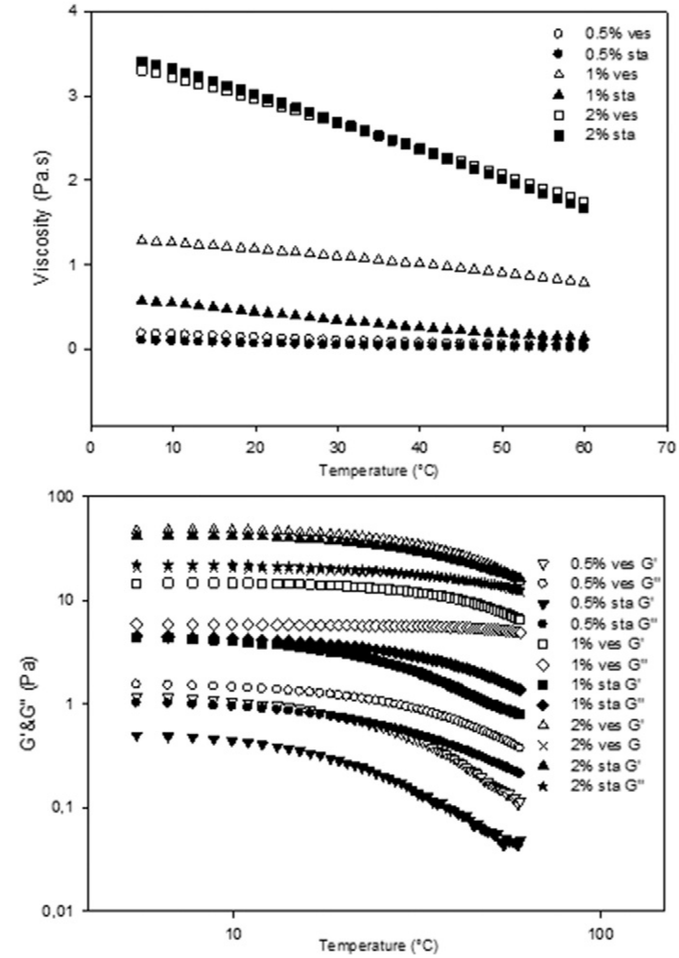

Fig. 3. Viscosity, Storage (G'), and Loss (G") modulus change of different gum solutions obtained from *X. axonopodis* pv. *vesicatoria* and *X. campestris* DSM 19000 against temperature.

strengthened by intermolecular and intramolecular hydrogen bonds [38]. At 0.5% concentration, both gums showed viscous nature as loss modulus was lower than the storage modulus. However, solution with 1% gum concentration from *X. axonopodis* pv. *vesicatoria* showed elastic nature unlike gum from *X. campestris* DSM 19000, which indicated higher gel-forming capacity of the novel type of xanthan gum. At 2%

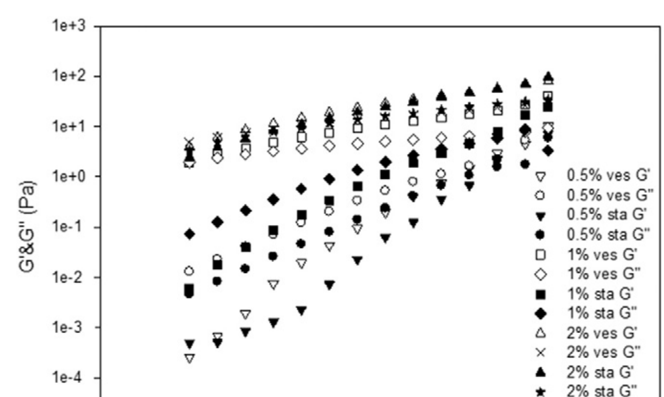

Fig. 4. Storage (G') and Loss (G") modulus change of different gum solutions obtained from *X. axonopodis* pv. *vesicatoria* and *X. campestris* DSM 19000 against angular frequency.

Table 2
Effect of xanthan gum concentration on G' (storage modulus) and G" (loss modulus) and R^2 (determination coefficient) values of different gum solutions obtained from different strains at 20°C.

Xanthan gum conc. (%)	Strain	K'	n'	R^2	K''	n''	R^2
0.5	*X. axonopodis* pv. *vesicatoria*	4.1*10^{-5}b	2.95a	0.99	2*10^{-6}b	3.62a	0.99
	X. campestris DSM 19000	0.076a	1.01b	0.95	5.24*10^{-3}a	1.51b	0.97
1	*X. axonopodis* pv. *vesicatoria*	5.95a	0.39b	0.98	3.8a	0.18b	0.99
	X. campestris DSM 19000	0.25b	0.99a	0.99	1.4b	0.34a	0.84
2	*X. axonopodis* pv. *vesicatoria*	18.04a	0.32a	0.99	11.21a	0.211a	0.99
	X. campestris DSM 19000	13.32b	0.42a	0.99	10.98a	0.259a	0.99

K' and K": consistency index for storage and loss modulus, respectively; n' and n": flow behavior index for storage and loss modulus, respectively; R^2: determination coefficient. Different lowercase letters show differences between the columns ($P < 0.05$).

concentration, viscoelastic behavior of the xanthan gum solutions was dominated by elastic nature, and G′ and G″ values of novel gum were higher than those of standard xanthan gum.

Oscillatory behavior of the solutions was also modeled according to the power law, and the corresponding viscoelastic parameters are shown in Table 2. *X. axonopodis* pv. *vesicatoria* showed weak gel-like behavior at all studied concentration as the slopes ($n' = 0.32$-2.95; $n'' = 0.18$-3.62) were positive and values of K' (4.1*10^{-5}-18) were much higher than those of K'' (2*10^{-6}-11) [39]. However, commercial xanthan obtained from *X. campestris* DSM 19000 only demonstrated weak gel-like behavior at high concentration (2%). At 0.5% concentration, both gums showed fluid-like behavior as their G" values were found to be higher than G' values [40].

Fig. 3 shows the comparison of temperature dependence of G' and G" between both xanthan gums. Remarkably, from 6°C to 60°C, G' and G" values of the novel xanthan gum solution from *X. axonopodis* pv. *vesicatoria* were always higher than those of the standard xanthan gum solution. Therefore, temperature stability of oscillatory behavior of the novel gum was also proved.

4. Conclusion

Because of wide applications of xanthan gum, it becomes important to develop a local strain of *Xanthomonas* that can produce the polysaccharide with high yield and technological properties. In this study, similar xanthan yields were obtained by the local isolate *X. axonopodis* pv. *vesicatoria* and the standard strain. Bacterial strain, agitation rate, and inoculum size were shown to affect the gum yield. For both strains, the best agitation rate and inoculum size conditions for the production of xanthan in an orbital shaker were found to be 180 rpm and 5%, respectively, which resulted in an average production of 10.96 and 11.19 g/L for *X. axonopodis* pv. *vesicatoria* and *X. campestris* DSM 19000, respectively. Increase in inoculum size and agitation rate lowered the xanthan yield by both microorganisms. Concerning the steady shear properties, the Ostwald-de-Waele model was used to compare the non-Newtonian behavior of the solutions; consistencies of solutions belonging to *X. axonopodis* pv. *vesicatoria* were higher at all concentrations. The Arrhenius model was used to compare the viscosity change in solutions with respect to temperature. Similar activation energies for both gum solutions indicated comparable temperature stability of the novel gum with the commercial xanthan gum. Regarding the viscoelastic properties of gum solutions, the power law was used to model dynamic oscillatory behavior of the solutions. Solutions belonging to *X. axonopodis* pv. *vesicatoria* showed weak gel-like behavior at all studied concentrations, whereas commercial xanthan obtained from *X. campestris* DSM 19000 demonstrated this behavior only at a high concentration (2%). Therefore, the results clearly indicated the better technological properties of the new gum synthesized from *X. axonopodis* pv. *vesicatoria,* and comparable yield values of both gums confirmed the suitability of industrial production by this organism. Further work will focus mainly on the chemical characterization of the polymer and on purification and clarification methods.

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