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Research Article

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Rheological behaviour and yield characterization of gum from local isolates: Xanthomonas hortorum pv. pelargonii and Xanthomonas axonopodis pv. begonia

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Abstract

Xanthan production capacity of local isolates *X. hortorum* pv. *pelargonii* and *X. axonopodis* pv. *begonia* were investigated by systematically changing fermentation conditions. Optimum yields were found as 11.19 g/L, 9.72 g/L and 9.65 g/L and for standard isolate *X. campestris* DSM 19000, *X. hortorum* pv. *pelargonii* and *X. axonopodis* pv. *begonia*, respectively. Optimum agitation rate and inoculum volume were found as 180 rpm and 5%. Moreover, better gel forming and thickening properties were obtained for xanthan gum from local isolates. Higher K value was observed for gum solutions of the local isolates at all concentration when Ostwald de Waele model was used. Activation energies changed between 4.85 and 25.43 kJ/mol and it is the highest for gum from standard isolate. Moreover, K' and K " values obtained from dynamic rheological analysis were higher for the local isolates than that of standard isolate. The results confirmed that the local isolates appeared to be suitable microorganisms for xanthan gum production.

Keywords: Xanthan gum, X. hortorum pv. pelargonii, X. axonopodis pv. begonia, Rheological behavior, Gum yield

Introduction

Xanthan gum is a natural and commercially important polysaccharide produced by isolates of *Xanthomonas* (Faria et al., 2010). Xanthan is widely used in food, cosmetic, oil recovery and pharmaceutical industries due to its ability to alter the rheological properties of aqueous solutions (Li et al., 2016).

Because of its wide applications, it becomes important to develop local isolate of *Xanthomonas* as a xanthan producer. It is known that different isolates of *Xanthomonas* can produce xanthan gums of different composition, viscosity and yield. The isolation and screening of *Xanthomonas* isolates from natural habitats is still the most efficient method of identifying isolates with high capacity of xanthan production and/or high rheological quality (Torrestiana et al., 1990; Antunes et al., 2003). It is necessary to investigate the rheological properties and chemical structure before deciding on their commercial

applicability. For this reason, there is a need to search for new isolates especially local isolates that can produce high yields of good quality xanthan gum.

In addition to bacterial isolates used, operational conditions during fermentation influence the yield, rheological properties and structure of xanthan gum produced (Garcia-Ochoa et al. 2004). Culture conditions like temperature, pH, inoculum size, agitation rate, air flow rate and medium composition are parameters that should be evaluated to optimize xanthan production, and improve rheological properties of the gum, mainly when wild isolates of *Xanthomonas* are studied.

Hence, in the present study, novel local isolates of *Xan-thomonas (Xanthomonas hortorum* pv. *pelargonii* and *Xan-thomonas axonopodis* pv. *begonia*) were evaluated in terms of gum rheology and xanthan gum production at different conditions of agitation rate and inoculum volume and compared

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with the *X. campestris* DSM 19000 standard isolate in order to determine the yield and quality of gums produced by the new isolate.

Materials and Methods Isolation and identification of microorganisms

X. axonopodis pv. *begonia* (Xcb-9), and *X. axonopodis* pv. *dieffenbachia* (Xad-2) were isolated from infected plant parts of begonia (Begonia X tuber hybrid), and anthurium (Anthurium andraeanum), respectively.

Identification of the isolates was initially confirmed by morphological, biochemical, and physiological tests including potassium hydroxide solubility for Gram reaction, catalase reaction, oxidative/fermentative metabolism, and hypersensitive reaction on tobacco leaves. Identification of the isolates was confirmed by fatty acid methyl ester (FAME) analysis. The two isolates of *Xanthomonas* spp. were isolated in Turkey (Aysan & Sahin, 2003; Mirik et al, 2007).

X. campestris DSM 19000 (NRRL B-1459) was obtained from the the The Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures (Germany).

Maintenance of microorganisms and cell growth

The microorganisms were maintained in YM (Yeast Malt) agar containing (gL⁻¹): 3.0 yeast extract; 3.0 malt extract; 5.0 peptone; 10.0 glucose; 20.0 agar and distilled water (pH 7.2) (Mesomo et al. 2009). Cultures were transferred at 2-week intervals. Cell production was performed in liquid media YM.

Xanthan gum production

The production of xanthan gum was carried out in 1000 ml Erlenmeyer flasks with 400 ml of medium consist of 40.0 (g/L) glucose, 2.1 (g/L) citric acid, 2.866 (g/L) KH₂PO₄, 0.507 (g/L) MgCl₂, 0.089 (g/L) Na₂SO₄, 0.006 (g/L) H₃BO₃, 0.006 (g/L) ZnO, 0.020 (g/L) FeCl₃·6H₂O, 0.020 (g/L) CaCO₃. Glucose was used as a carbon source for the fermentation studies (Liakopoulou-Kyriakides et al., 1999). The fermentations were commenced with inoculums size of 5% (v/v) and 10% (v/v), experiments were conducted at four different agitation rates (180, 200, 220 and 300 rpm) on a orbital shaker (STUART SI500). As showed in the review of Rosalam and England (Rosalam & England, 2006), several works indicated the optimal conditions as temperature at 28°C, fermentation time of 72 h and initial pH at 7.2. Based on the results presented in the literature, during the process, the temperature of the system was maintained at 28°C. This control was necessary since heat was released during the substrate consumption reactions, and thus 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. Runs were terminated after 72 h of incubation. All experiments were performed duplicate.

Recovery of xanthan gum

The fermented broth was centrifuged 30 min for cell removal (SIGMA 2-16KL) at 4 °C and 10.000 rpm. Isopropanol (Merck) was added to the supernatant in the proportion of 1:3 (v/v) for precipitate of the biopolymer. The mixture was stored at 4 °C for 24 h and then centrifuged again at 10.000 rpm for 30 min at 4 °C to recover the precipitated gum, which was dried in an oven at 50 °C until constant weight to determine the xanthan gum content. The production of the biopolymers of each isolate at different conditions was evaluated by the weight of the dry product per liter of fermented broth and the averages expressed in g/L.

Rheological analysis of the xanthan gum

Frequently used concentrations in food systems (0.5%, 1%, and 2%) were used in all the rheological measurements. 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 hr 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.

Steady shear measurements

All rheological measurements were conducted using a controlled stress Discovery Hybrid Rheometer-2 (TA Instruments New Castle, DE, USA) fitted with a parallel-plate geometry (stainless steel, 40 mm diameter, 1000 μ m gap). Shear rate range of 1-100 s⁻¹ was used for xanthan solutions at 0.5, 1 and 2 wt % and shear rate, shear stress, normal force, torque and apparent viscosity data were collected during experiments. Ostwald de Waele model was fitted to flow behaviors of the samples;

$\sigma = K(\gamma)^n$ (1)

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

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 conducted to determine linear viscoelastic region. Frequency sweep test was performed at 0.6 Pa over a frequency range of 0.05–100 rad/s. The elastic or storage modulus (G') and the viscous or loss modulus (G'') were modeled by a power law;

$G'=K'(\omega')^{n'}$ (2)

 $G''=K''(\omega'')^{n''}$ (3)

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

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. $A=A_0\exp(E_0/RT)$ (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).

Statistical analysis

Statistical analyses were conducted using Minitab for Windows Release 14[®]. Standard errors were calculated using the Duncan's multiple range test.

Results and Discussion

Effect of Agitation Rate and Inoculum Volume on Xanthan Gum Production The results of experiments conducted in shake flasks in order to evaluate the effects of agitation rate and inoculum volume on the production of xanthan gum by X. hortorum pv. pelargonii, X. axonopodis pv. begonia and X. campestris DSM 19000 were shown in Figure 1-3. Based on the results of the experiments, both inoculum volume and agitation rate have been shown to be important factors, for xanthan concentration. Optimal xanthan production was found with agitation at 200 rpm and inoculum volume at 5 % for isolate X. hortorum pv. pelargonii as a yield of 9.72 g/L (Fig. 1). The corresponding optimum agitation rate and inoculum volume for other isolate X. axonoopodis pv. begonia were 180 rpm and 5% respectively, which resulted in a xanthan production of 9.65 g/L (Fig. 2). Similarly, the greatest production of 11.19 g/L for the isolate X. campestris DSM 19000 was found with agitation at 180 rpm and inoculum volume at 5%, corresponding to the central point, as can be observed in Fig 3. Generally, X. campestris DSM 19000 exhibited higher ability for xanthan gum production as compared to isolates at different conditions.

The results of previous studies (Torrestiana et al., 1990; Nitschke & Thomas, 1995; Sánchez et al., 1997; Souza & Vendruscolo, 1999) showed that the production depended on the isolate and that was also confirmed in this work. It can be deduced, therefore, that the selection of isolate should be the first stage in the xanthan search for the highest yield.

In inoculum experiments, the aim is to optimize the cell concentration to give maximum xanthan production. When xanthan gum production values were compared at 5% and 10% inoculum size of Xanthomonas species, higher yields were obtained for all microorganisms at the rate of 5% inoculum except for studies with 300 rpm. The inoculum size 10% might facilitate better the production of biomass than xanthan. High amount of inoculum was not good enough for the production as the nutrients and the space for them was not sufficient to grow actively. Depending up on the isolates, the inoculum size could vary. Papagianni et al. (2001) study xanthan production using 10% inoculum volume. However, Leela and Sharma (2000) and Fernandez-Silva et al. (2009) used 2-15% and 20% inoculum volumes, respectively for xanthan production. Our findings were in agreement with those obtained by Ben Salah et al. (2011), who observed maximum xanthan yield at 5% inoculum.

Furthermore, the graphs of Fig. 1-3 showed that, in general, agitation had a significant effect on xanthan production. Xanthan production was partly associated with metabolic growth; up to 180 rpm, an increase in xanthan production occurred due to oxygen transfer limitation. However, the xanthan yield dropped when higher rate from 220 rpm was applied, probably due to cellular damage by hydrodynamic stress. Agitation speed could be beneficial to the growth and performance of the microbial cells by improving the mass transfer characteristics with respect to substrates, products and oxygen (Garcia-Ochoa et al., 2000). Thus, agitation resulted in a better mixing of the fermentation broth, allowing maintaining a concentration gradient between the interior and the exterior of cells.

Some researchers reported that higher stirrer speed was necessary for xanthan production by *X. campestris* pv. *mangif*-

eraeindicae IBSBF 1230 (Mesomo et al., 2009), *X. arboricola* pv *pruni* 106 (Borges et al., 2009), *X. campestris* ATCC 33913 (Psomas et al., 2007), *X. campestris* PTCC 1473 (Gilani et al., 2011), *X. campestris* (Casas et al., 2000), *X. campestris* ATCC 1395 (Papagianni et al., 2001). Nevertheless, our results were in agreement with that of Ben Salah et al. (2011) 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.

Our results showed that; there is an optimum agitation rate which can not cause bacterial damage and at the same time not limit mass transfer for each bacteria used in xanthan gum production. These results confirmed that yield depended on fermentation parameters and a type of bacterial isolate.

Rheological properties of xanthan gums Steady shear properties

The gums produced by X. axonopodis pv. begonia (Xcb-9), X. axonopodis pv. pelargonii (Xad-2) and standard isolate (X. campestris DSM 19000) were also evaluated rheologically and Ostwald de Waele model was used to fit experimental viscosity versus shear rate data to make comparison of non-Newtonian behavior of the solutions (Table 1). Determination coefficient values were higher than 0.98 indicating good fitting of the model and pseudoplastic behavior of gum solutions. Generally solutions of exopolysaccharides obtained from microorganisms showed shear thinning behavior (Kim & Yoo, 2011). The flow behavior index (n), obtained from the model equation, indicated the extent of shear thinning (pseudoplastic) behavior as it deviated from 1. At all gum concentrations of the solutions, gum from standard isolate (X. campestris DSM 19000) showed higher n value than those from the local isolates, indicating more pseudoplastic behavior of local isolates. This showed clearly different morphology of gums obtained from different isolates. Lee and Chang (2015) reported that structure of polysaccharides which inhibits the formation of intramolecular hydrogen bonding kept molecule in the extended form during the application of shear. Therefore, solution showed shear thinning behavior. However, when intramolecular hydrogen bonding was formed due to different branch groups in a molecule, it caused lower shear thinning behavior. This rheological feature is also desired property of xanthan solutions as it increases organoleptic qualities in food and causes high degree of mixability, pumpability, and pourability during their processing and/ or actual use (Ki-Won et al., 2006). This showed that biopolymers obtained biotechnologically from Xanthamonas species isolated from begonia and anthurium plant would be a more suitable thickener or an efficient stabilizer for suspensions and emulsions in many industries (Garcia-Ochoa et al., 2000). As could be seen from K (consistency index) values, gum from local isolates formed higher viscosity solutions at all concentrations than standard isolate indicating that new gums had higher branching ratio as well as their greater hydration capacity and thickening properties.

Concerning the effect of temperature on the viscosity values of gum solutions, Arrhenius model was used. R^2 values were found between 0.96 and 0.99 as shown in Table 2. It was clearly seen that activation energies changed between 4.85 and

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25.43 kJ/mol. At all concentrations activation energy of gum from standard isolate was higher than gums from local isolates indicating better temperature stability by lower viscosity decrease as temperature increased. Generally high temperature of solution increased intermolecular distances and decreased chain overlap and entanglement which resulted in viscosity decrease. A similar decrease in temperature was also observed in other polysaccharides such as pectin, starch and gums (Hosseini-Parvar et al., 2010).

Dynamic rheological properties

Gel properties of gums can be determined by investigating viscoelastic properties of gum solutions. Dynamic rheological behavior of the solutions was modeled according to power law and the corresponding viscoelastic parameters were shown in Table 3. *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) [32]. 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 the value of the exponents describing the dependence of moduli with frequency, being higher than unity (Martinez-Padilla et al., 2004). *K'* values from local isolates were significantly higher than that of standard isolate for all concentrations except 0.5%. This showed that better gel quality of gum solutions obtained from local isolates, *X. axonopodis* pv. *begonia* and *X. hortorum* pv. *pelargonii.*

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

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Xanthan gum conc. (%)	Isolate	K (Pa s ⁿ)	n (-)	R^2
0.5	X. axonopodis pv. begonia	0.452a	0.55±0.1b	0.99
	X. hortorum pv. pelargonii	0.335a	0.532b	0.99
	X. campestris DSM 19000	0.154b	0.688a	0.99
1	X. axonopodis pv. begonia	6.321a	0.185c	0.99
	X. hortorum pv. pelargonii	2.155b	0.363b	0.99
	X. campestris DSM 19000	1.445b	0.457a	0.98
2	X. axonopodis pv. begonia	22.872a	0.171b	0.99
-	<i>X. hortorum</i> pv. <i>pelargonii</i>	19.27b	0.183b	0.99
	X. campestris DSM 19000	16.295c	0.236a	0.99

K: consistency index; *n*: flow behavior index; R^2 : determination coefficient Different lowercase letters show differences between the columns (P<0.05).

Table 2. Activation energies of xanthan gum obtained from different isolates with different gum concentrations

Xanthan gun (%)	n conc.	Isolate	A_0 (Pa s ⁿ)	Activation energy (kJ/mol)	R^2
0.5		X. axonopodis pv. begonia X. hortorum pv. pelargonii	2.03*10 ⁻⁵ b 0.0001a	21.21b 17.7c	0.98 0.98
		X. campestris DSM 19000	1.98*10 ⁻⁶ b	25.43a	0.99
1		X. axonopodis pv. begonia	0.001b	15.85b	0.96
		X. hortorum pv. pelargonii	0.17a	4.85c	0.96
		X. campestris DSM 19000	7.4*10 ⁻⁵ c	21.25a	0.98
2		X. axonopodis pv. begonia	0.15a	7.17b	0.97
		X. hortorum pv. pelargonii	0.24a	6.36b	0.97
		X. campestris DSM 19000	0.046b	10.12a	0.97

A: constant determined from the Arrhenius relationship; R^2 : determination coefficient

Different lowercase letters show differences between the columns (P<0.05)

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Table 3. Effect of xanthan gum concentration on G' (storage modulus), G'' (loss modulus), R^2 (determination coefficient) values
of different gum solutions obtained from different isolates at 20°C

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Xanthan gum conc. (%)	Isolate	K'	n'	<i>R</i> ²	<i>K"</i>	<i>n</i> "	R ²
0.5	X. axonopodis pv. begonia	0.055b	1.192b	0.98	0.069b	0.98a	0.94
	X. hortorum pv. pelargonii	0.0013c	2.043a	0.99	0.362a	0.355b	0.84
	X. campestris DSM 19000	0.076a	1.01b	0.95	5.24*10 ⁻³ c	1.51c	0.97
1	X. axonopodis pv. begonia	6.943a	0.266c	0.99	3.077a	0.183c	0.98
	X. hortorum pv. pelargonii	0.560b	0.798b	0.99	1.415b	0.418a	0.99
	X. campestris DSM 19000	0.25b	0.99a	0.99	1.4b	0.34b	0.84
2	X. axonopodis pv. begonia	29.323a	0.254b	0.99	13.835a	0.172b	0.99
	<i>X. hortorum</i> pv. <i>pelargonii</i>	23.99b	0.290b	0.99	12.770a	0.180b	0.99
	X. campestris DSM 19000	13.32c	0.42a	0.99	10.98b	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).



Figure 1. Effects of inoculum volume and agitation rate on the production of xanthan for X. hortorum pv. pelargonii (g/L)







Figure 3. Effects of inoculum volume and agitation rate on the production of xanthan for X. campestris DSM 19000 (g/L)

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Conclusion

Rheological behavior of xanthan produced from X. hortorum pv. pelargonii and X. axonopodis pv. begonia isolates and standart isolate X. campestris DSM 19000 was characterized and the effect of agitation levels and inoculum volume on xanthan gum yield was investigated. It was determined that the xanthan gum production potentials of the isolates used in this study were high and the xanthan yields obtained with the standard isolate X. campestris DSM 19000 were close to the results obtained with the isolates. The optimised conditions for the production of xanthan in terms of agitation and inoculum were 200 rpm and 5 % for X. hortorum pv. pelargonii and 180 rpm and 5 % for X. axonopodis pv. begonia, resulting in a mean production of 9.72 and 9.65 gL⁻¹ gum respectively, in 72 h. Generally, it was found that studied isolates were fragile to high agitation rates.

Concerning the rheological behavior, shear thinning properties were observed for all gums. Gums form local isolates caused higher consistency of solution when used at same concentrations compared to gums from standard isolate. Temperature stability of solutions prepared by standard isolate was better. However, gel forming capacity of gums from local isolates, X. hortorum pv. pelargonii and X. axonopodis pv. begonia was observed to be better than standard isolates.

Rheological properties and vield values confirmed that isolate X. hortorum pv. pelargonii and X. axonopodis pv. begonia appeared to be suitable microorganisms for xanthan gum production when compared to standard isolate, X. campestris DSM 19000.

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