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Semi-pilot production of xanthan gum using *nejayote* as culture medium substrate

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Abstract

Background: Xanthan gum is an industrialized polysaccharide produced by *Xanthomonas* genus. Alternative carbon sources for *Xanthomonas* culturing may help reducing its production cost. *Nejayote*, a residue from maize nixtamalization process, is an alternative culture medium substrate to produce xanthan. In this study, industrial and semi-industrial *nejayote* alone or in combination with supplements, were tested for xanthan production by *Xantomonas campestris* (1×10^8 cells/mL inoculum), using YGC medium as control, in 100 mL and 1 L volumes. Cellular growth was determined by the colorimetric MTT reduction assay (OD₅₄₀). Xanthan pyruvate and acetyl groups from *nejayote* plus supplement cultures in the bioreactor were evaluated (OD₅₇₀).

Results: Industrial *nejayote* steadily produced up to 4 g/L xanthan, as compared with YGC medium control, which increased its production over time up to 9.3 g/L at 96 h. Cellular activity assay revealed that the highest values after 24 h (3.88 and 2.71 OD₅₄₀ for YGC and industrial *nejayote*, respectively). *Nejayote* supplemented with MgSO₄•7 H₂O resulted in the highest xanthan production (10.8 g/L), but low cell growth (3.6 and 1.82 OD₅₄₀ for YGC and *nejayote* plus supplement, respectively), after 96 h of culture. Furthermore, gum yield reached up to 6 g/L and 1.9 OD₅₄₀, after 96 h of *nejayote* plus supplement culture, using a 14 L bioreactor. Xanthan pyruvate and acetyl groups from *nejayote* plus supplement cultures in the bioreactor resulted in similar amounts (0.107 and 0.130 OD₅₇₀, respectively), compared with a commercial biopolymer (0.148 and 0.127 OD₅₇₀ respectively).

Conclusions: *X. campestris* effectively grew and produced industrial-quality xanthan gum in *nejayote* substrate-containing culture medium, thus providing an inexpensive alternative for bioremediation.

Key words: YGC medium, pyruvate and acetyl groups, bioremediation, *Xanthomonas campestris*

Background

Xanthan gum is the most industrialized polymer worldwide, mainly produced by the bacterium *Xanthomonas campestris*. It has broad applications in different industries for its potential to alter viscosity in aqueous solutions, in addition to be an excellent stabilizer in a variety of suspensions, emulsions, and foams, remaining stable in ample ranges of temperature, pH, and salt concentrations (Velu et al., 2016). To supply the various consumer sectors, 86,000 tons of xanthan gum are produced each year at an approximate cost of \$ 400 million dollars (Kalogiannis et al., 2003; De Monaco-Lopes et al., 2015).

Xanthan is aerobically produced at temperatures ranging from 27 °C to 30 °C. In the commercial production process, *X. campestris* converts about 70% of the substrate (mainly sucrose, glucose, and citric acid) to xanthan, using a batch culture process, followed by heat treatment, gum removal from cells, alcohol recovery, drying, and grinding (De Monaco-Lopes et al., 2015).

In regard to substrate production, Panyu et al. (2016) emphasized that because of glucose and sucrose high costs (US \$ 400-600/ton), the produced gum results expensive (US \$ 4,000-5,000/ton), for which it is important to search and test inexpensive alternative substrates (Katherine et al., 2017). From economic and environmental perspectives, the use of substrates with high organic matter from agricultural or industrial production residues, is desirable (García-Depraect et al., 2017). In recent decades, the use of agro-industrial waste

as bacterial carbon source has been evaluated (De Monaco-Lopes et al., 2015). According to Gunasekar et al. (2014), several low-cost substrates, including whey or tapioca pulp, have been used for the effective scaled-up xanthan production process, resulting in yields of up to 16.4 and 7.1 g/L, respectively.

At laboratory level, the use of cassava bagasse, hydrolyzed by acid and supplemented with nitrogen sources, was reported to be a suitable substrate for xanthan production by *X. campestris*, resulting in an average gum yield of 14 g/L (Woiciechowski et al., 2004). Similarly, xanthan fermentation by *X. campestris* strain NRRL-B-1449 from sugar cane molasses, using different nitrogen sources, was evaluated at continuous batch level. Gum production was estimated within 24 h interval, where the results showed a maximum yield of 3.6 g/L, after 120 h incubation (Kalogiannis et al., 2003). Niknezhad et al. (2015) obtained 16.4 g/L of xanthan by *X. campestris* from 65.2 g/L cheese whey serum (characterized by a high organic content and posing a severe risk to the environment), as a carbon source after 48 h of culture.

At industrial level, nixtamalization is a process in which corn kernels are cooked in a saturated calcium hydroxide solution and after wet grinding, they are hydrated to extract starches. Next, they are cooked under alkaline treatment (0.6% to 1.2% calcium hydroxide with regard to corn weight), during a period of 50 to 70 min at 90-95 °C, followed by a resting time of 12 h to 14 h. After this, draining and washing are performed to remove calcium excess and discard dry matter. The obtained material, named nixtamal, is ready for wet grinding, resulting in useful dough to produce nixtamalized corn flour that is hydrated to make tortillas and a wide range of snacks (Olguín-Arteaga et al., 2015). During nixtamalization, large volumes of *nejayote* are generated. It has been estimated that a corn-processing plant, producing nixtamal, with a capacity of 600 tons per day, generates

between 1,500 and 2,000 m³ of *nejayote*. In Mexico alone, the annual generation of this by-product reaches 14.8 billion liters (García-Depraect et al., 2017).

During nixtamalization process, high levels of solid materials, consisting of corn dry matter, are usually lost. Based on organic and inorganic compounds content, described residues represent contamination factors. This effluent is considered polluting due to its high pH (12 to 14) (Salmerón-Alcocer et al., 2003) and organic matter load (2,540 mg/L) (Valderrama-Bravo et al., 2012). In fact, because of the elevated number of organic residues, *nejayote* cannot be directly integrated in the wastewater or even drenched into soil (Valderrama-Bravo et al., 2012; Meraz et al., 2016). These production losses are economically significant for a manufacturer due to mass loss, waste processing, and disposal costs, as well as environmental contamination, and potential legal penalties (Rosentrater, 2006).

According to Gutiérrez-Urbe et al. (2010), *nejayote* contains a high amount of corn solids, and because of its nutritional content (polysaccharides, raw fiber, starch, proteins, vitamins, and phytochemicals), it has been suggested for biogas production, probiotics culture, and microbial culture media formulation (Figueroa-González et al., 2011; García-Depraect et al., 2017). Furthermore, the *nejayote* biological oxygen demand/chemical oxygen demand (BOD/COD) proportion (0.6), suggests that the organic matter is potentially biodegradable (García-Depraect et al., 2017).

Since *Aspergillus awamori* produced enzymes with amylolytic activity after incubation in *nejayote*, Domínguez-Espinosa and Pacho-Carrillo (2003) inferred that such compounds supported effluent degradation. García-Depraect et al. (2017) studied biohydrogen (bioH₂) production in a discontinuous reactor from the co-digestion of *nejayote*, evaluating the effects of different proportions of residues, reporting that the microbial

structure of the inoculum allowed adaptation for feeding on substrates. Based on this and the different protocols to treat *nejayote*, the use of this residual effluent to reduce contamination levels and obtain a value-added product, such as xanthan gum, should be evaluated.

Nejayote represents a potential culture medium substrate to be used in xanthan production, based on *X. campestris* metabolic rate. It then becomes an alternative for reducing production costs by optimizing this bioprocess and facilitating waste re-incorporation into soil or use in other processes, because of the metabolic modifications made by this microorganism. Therefore, the aim of the present study was to determine feasibility and optimization of xanthan gum production up to semi-pilot level, using *nejayote* as substrate.

Results

X. campestris xanthan gum production and cellular activity using industrial *nejayote*

Similar xanthan production was observed at 24 h of culture in *nejayote* and YGC broth. However, gum generated in *nejayote*-based medium did not increase over time, obtaining results not greater than 4 g/L at the end of the test. In contrast, gum production in YGC broth continued to increase during 96 h up to 9.3 g/L (Fig. 1). Cellular activity using *nejayote* as culture medium substrate, showed an exponential rise during the first 24 h, which decreased over time, as compared with that using YGC medium, maintaining activity

during the incubation time. After 24 h of culture, the highest ODs were 3,884 and 2,713 by control medium and industrial *nejayote*, respectively (Fig. 1).

Physicochemical characteristics of xanthan from industrial *nejayote*

Physicochemical parameters analysis results of xanthan obtained from industrial residues demonstrated compliance with NRF-300-PEMEX-2012 Standard specifications for humidity and viscosity, showing values of 5.90% and 24.0 cP (centipouls) with a reading at 600 r/min, respectively. Nevertheless, particle sizes did not satisfy the standards, since their dimensions were below the required specifications (Table 1).

Xanthan production and *X. campestris* activity in semi-industrial *nejayote* cultures

After evaluating 100 mL of medium at 24 h of culture, production was higher in the *nejayote* medium (2.56 g/L yield) alone or supplemented with calcium carbonate (4.20 g/L yield) and hepta-hydrated magnesium sulfate (3.66 g/L yield), than that of YGC medium (2 g/L yield), maintaining similar production values until the end of the fermentation time, whereas bacteria kept an exponential increase in YGC medium (Fig. 2A). Furthermore, xanthan biosynthesis developed up to 48 h in treatments supplemented with yeast extract and $\text{CaCO}_3 + \text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$. Bacteria cultured in residue treatments with yeast extract + CaCO_3 , yeast extract + $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, and yeast extract + $\text{CaCO}_3 + \text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ did not produce gum (Fig. 2A). The treatment that showed the best results in terms of biopolymer production was *nejayote* supplemented with CaCO_3 , producing 5.7 g/L after 120 h of culture, which was lower compared with that of YGC medium (7.36 g/L).

Most treatments showed increased bacterial activity during the first 48 h with ODs by *nejayote* + CaCO₃ treatment and YGC medium of 3.548 and 3.773 respectively. Bacteria metabolic activity decreased over time, among *nejayote* treatments, but it was stable in YGC medium until the end of the experiment, similar to *nejayote* treatment supplemented with yeast extract, in which cellular activity increased during 72 h (OD₅₄₀ = 3.787) (Fig. S1A).

In addition, semi-pilot (14 L) xanthan production was developed from semi-industrial *nejayote* treatments (using medium volumes up to one liter), showing gum production, in which after 24 h culture, xanthan production was higher among *nejayote* treatments, obtaining up to 5 times higher yields (5.7, 9.96 and 6.4 g/L yields in 14 L) by *nejayote* alone or supplemented with MgSO₄ • 7 H₂O or CaCO₃, respectively, compared with that of control medium (1.93 g/L yield). At the end of the experiment (96 h), *nejayote* supplemented with MgSO₄ • 7 H₂O reached 11.46 g/L gum production, compared with 4.16 g/L by YGC medium (Fig. 2B).

During the first 24 h *X. campestris* cellular activity evaluation revealed that in control medium (YGC), cell activity was higher (OD₅₄₀ = 3.795) compared with that of *nejayote* treatments, followed by *nejayote* + CaCO₃ (OD₅₄₀ = 2.639). Bacteria metabolic activity in *nejayote* + CaCO₃ increased for 48 h and remained constant until the culture ended, whereas in control medium it started the stationary phase after 24 h of culture. After 72 h, cellular activity of all other treatments remained constant until the end of the experiment, showing lower cellular activity as compared with positive control (Fig. S1B).

Xanthan gum produced in *nejayote* culture

After xanthan gum produced in *nejayote* was analyzed, the positive control had characteristics that clearly differentiate it from the rest of the samples, such as a larger size, as well as a spongier consistency and cleaner appealing (white coloration). Xanthan gum produced in the semi-industrial *nejayote* treatments, had smaller size and were more compact with harder consistency compared with control, before and after drying, in addition to a darker coloration (brownish). The gum from industrial *nejayote* resulted in an even darker coloration (light brown) and after dried, it lost its spongy characteristic and resulted in an even more compact product (Table S1).

Statistical analysis

Xanthan production using YGC medium was significantly higher (8 g/L yield) ($F_{4,5} = 87,068$; $p < 0.05$), compared with that of industrial *nejayote* culture (3 g/L yield) (Fig. 3A). Experiment using semi-industrial treatments as substrate in 100 mL volume, showed gum production using *nejayote* without supplements of 5.1 g/L, which was significantly lower than the positive control ($F_{18,26} = 144,598$; $p < 0.05$), whereas production in *nejayote* supplemented with CaCO_3 was even higher (5.7 g/L) (Fig. 3C).

Results from experiments using 1 L volume semi-industrial treatments as substrate differed from previous experiments, where gum production in control medium decreased 50% to achieve 3.86 g/L yield, which was significantly lower compared with *nejayote* treatments and the use of $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ showed the highest production (10.83 g/L) at the end of the experiment ($F_{5,12} = 53,330$; $p < 0.05$). Similar results were observed in *nejayote* + CaCO_3 treatment, compared with previous tests, with a production of 6.06 g/L (Fig. 3D).

Cellular activity (OD_{540}) using YGC medium was significantly ($F_{4,5} = 660,080$; $p < 0.05$) higher (more than double) compared with that of *nejayote* medium ($OD_{540} = 3,636$ and 1,506 respectively) (Fig. 3B). For the 100 mL volume experiment, semi-industrial *nejayote* treatments presented the highest OD_{540} when the culture was ended, compared with treatments supplemented with yeast extract, yeast extract + $CaCO_3$ + $MgSO_4 \cdot 7 H_2O$, and yeast extract + $CaCO_3$, with A_{570} of 3,198, 2,933, and 2,838 respectively, which were significantly ($F_{18,26} = 37,636$; $p < 0.05$) lower than those of YGC medium (3,750) (Fig. 4A).

Cellular activity in 1 L volume experiments with *nejayote* was similar than that in 100 mL experiments, however *nejayote* supplemented with yeast extract produced more gum in 100 mL ($OD_{540} = 3,198$) volume experiments than in 1 L ($OD_{540} = 2,154$). *Nejayote* supplemented with $CaCO_3$ showed significantly ($F_{5,12} = 72.094$; $p < 0.05$) higher OD_{540} (2.786) compared with all other treatments with *nejayote*, although still lower compared with that YGC medium (Fig. 4B).

Xanthan-covalently linked pyruvate and acetyl groups content

Pyruvate group content was determined in semi-industrial *nejayote* samples from 100 mL and 1 L volume experiments (Fig. 5A). In both experiments, pyruvate content in *nejayote*-based treatments was similar compared with control (YGC) ($F_{8,18} = 68,334$; $p > 0.05$), as well as among experiments ($F_{8,18} = 77.076$; $p > 0.05$). OD_{570} values were similar in YGC medium compared with commercial gum sample (0.153 and 0.148, and 0.131 and 0.148 in 100 mL and 1 L volume experiments, respectively), but lower compared with commercial pyruvate-enriched medium as control (0.175) (Fig. 5A).

Acetyl groups content analysis showed no significant OD₅₇₀ differences among *nejayote*-based treatments between 100 mL ($F_{6,14} = 81.749$; $p > 0.05$) and 1 L experiments ($F_{6,14} = 61.581$; $p > 0.05$); nevertheless, they were lower than those of *nejayote* without supplements (OD₅₇₀ = 0.056) in 1 L volume experiments (Fig. 5B).

***X. campestris* cellular activity and xanthan production in semi-industrial *nejayote* at semi-pilot level**

After testing *X. campestris* xanthan production at semi-pilot level, an exponential increase in gum production and cell activity was observed during the first 16 h of culture in *nejayote* supplemented with MgSO₄ • 7 H₂O treatment, with gum values of 3.2 g/L and OD₅₄₀ = 1.554, respectively. Xanthan production and cellular activity remained constant for 72 h of culture, where the maximum production values were 4.2 g/L and OD₅₄₀ = 1.902, respectively, after 80 h (Fig. 5B).

Xanthan-covalently linked pyruvate and acetyl groups content at semi-pilot level

Gum sample produced by *X. campestris* in the bioreactor showed a pyruvate content comparable ($F_{4,2} = 3.522$; $p > 0.05$) to that from commercial xanthan, with OD₅₇₀ values of 0.107 and 0.148 respectively (Fig. 6). Similarly, acetyl groups content values were no different ($F_{4,3} = 0.720$; $p > 0.05$) from *nayajote* (OD₅₇₀ = 0.13) and commercial (0.127) gum, respectively.

Discussion

Different classes of carbon sources have been reported to produce xanthan gum, but carbon concentration generally affects sugar conversion to polysaccharide yield (Kennedy & Bradsahw 1984; Woiciechowski et al., 2004). *Nejayote* is an important carbon source (high carbohydrates and reducing sugars content) for microorganisms in bioprocesses, where the degradative potential mainly depends on substrate availability (García-Depraect et al., 2017; Acosta-Estrada et al., 2014). In our study, *X. campestris* grew and produced xanthan gum using industrial *nejayote* as culture medium, but its growth decreased after 24 h of culture (Fig. 1), probably due to a reduction in the carbon source availability or completely nutrient consumption, negatively affecting its cellular activity. Nevertheless, xanthan gum production in the first 24 h of culture was comparable with that of reference medium (2.86 g/L versus 3 g/L in *nejayote* and YGC broth, respectively) (Fig. 1). Gum yield in *nejayote*-based medium remained constant, with a final production of 4 g/L, whereas in YGC medium the final yield reached 9.3 g/L.

In this study, xanthan produced in *nejayote* yielded close to 4 g/L and 5 g/L in industrial and semi-industrial waste, respectively, which is desirable for a waste material (Li et al., 2016). In most cases, in order to produce this gum using alternative substrates, it is required the addition of supplements as nutrients. Katherine et al. (2017), reported xanthan gum production by *X. campestris* strain NCIM 2961, using jackfruit seed powder as substrate, reaching 51.62 g/L yield, concluding that seed powder optimum level was 4 g/L, however, the culture medium was supplemented with peptone, citric acid, K_2HPO_4 , and KH_2PO_4 , increasing production costs.

Lopez et al. (2001) tested *X. campestris* strains to evaluate xanthan production from industrial olive mill wastewaters as culture medium supplement. They reported optimum xanthan yields of 4 g/L and 2 g/L after adding 40% and 20% residue to *X. campestris* T646 and B-1459 S4LII strain cultures, respectively.

Using cassava bagasse hydrolyzate as a substrate (starch residue), in combination with several nitrogen sources, Woiciechowski et al. (2004) produced an optimum gum yield of 14 g/L, which was higher than that (4.83 g/L yield) obtained with *nejayote* (semi-industrial) supplemented with yeast extract as a nitrogen source in 100 mL and 6.46 g/L in 1 L volume experiments at the end of the experiment. However, *X. campestris* did not produce xanthan gum in *nejayote* supplemented with potassium nitrate (Fig. 2A). Similar results were reported by Moshaf et al. (2011), using *X. campestris* strain PTCC1473, date palm residues as culture medium, and the response surface method; according to the statistical model, they concluded that lower nitrogen level led to higher xanthan production. This may explain why gum production in *nejayote* treatment supplemented with yeast extract (high nitrogen concentration) in our study, did not correlate with higher bacteria metabolic activity compared with reference medium (Fig. S1A).

Gunasekar et al. (2014) produced xanthan gum in pre-treated tapioca pulp with different concentrations of sulfuric acid, where the maximum yield was 7.1 g/L after using 0.5% acid concentration pre-treatment. Gum production was similar to the maximum xanthan yield in *nejayote* supplemented with CaCO₃ treatment (7.6 g/L) in 1 L volume experiments of our study. Although acid pre-treatment may help to metabolize the hydrocarbon polymers present in an organic waste, high concentrations of acid increases formation of inhibitory substances, which decreases gum production and affects bacteria energy, thus inhibiting and damaging their biological activities (Taherzadeh et al., 2000; Li

et al., 2016). In addition, xanthan viscosity proportionally decreased with increased acid concentration, therefore it is important to evaluate and determine the optimal acid concentration to avoid losing gum yield and product quality.

After inoculation, Li et al. (2016) reported that culture started an exponential growth phase after 2 h and entered a stationary phase around 22 h; after 38 h it remained unchanged, agreeing with the results obtained from cellular activity using the positive control of YGC broth, where the stationary phase began after 24 h and remained constant until the culture was culture ended. In contrast *X. campestris* culture in the semi-industrial *nejayote* treatments remained constant after 48 h (Fig. S1A), in accordance with the time in which xanthan stopped being produced, this for *nejayote* without additives or supplemented with $\text{CaCO}_3 + \text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, or yeast extract and $\text{CaCO}_3 + \text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ treatments. This may be partially explained due to physical and nutrient limitations within the culture system, where its viscosity may limit gas exchange and nutrients availability (Woiciechowski et al., 2004). Viscosity rise in the culture medium, related to the amount of produced gum, interferes with oxygen transfer, which reduces or stop microbial activity, even if a carbon source is available in the medium.

In our study, xanthan production was scaled at a semi-pilot level, where volumes up to 1 L were initially tested, in order to determine if this bioprocess could be carried out at higher volumes. After scaling from 100 mL to 1 L volume, results showed higher gum production by *X. campestris* in YGC medium using lower volume (7.36 and 3.86 g/L respectively), whereas cellular activity remained equivalent. We believe such values are the result of a significant increase in viscosity and decrease in the gas-liquid mass transfer rate (oxygen transfer rate) (Casas et al., 2000), which could clearly lead to a reduction of gum production.

Nevertheless, xanthan yields from *nejayote* plus supplements were similar or higher than *nejayote* alone. *Nejayote* supplemented with $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ showed the highest production and was selected for the bioprocess scaling at bioreactor level, where production yielded 5.53 g/L, which was lower than experiments using lower volumes. Rosalam & Englan (2006) reported that different growth phases and perhaps a culture medium alteration, such as a higher non-filterable solids amount, like *nejayote*, limited nutrients, thus reducing yield, but not the produced xanthan quality.

Results from pyruvate and acetyl groups content showed that gum samples from different treatments and assays possessed both groups. Provided values could help to infer about some of the xanthan rheological properties (Lopes et al. 2015), where an apparent low viscosity compared with commercial samples was related to lower pyruvate group levels. Similarly, a high acetyl content was related to xanthan samples with high molecular weight and therefore, producing high viscosity solutions (De Monaco-Lopes et al., 2015). However, levels of acetyl and pyruvate substitution vary with growth conditions among other parameters (Becker, 1998); therefore, it is recommended to perform more assays with *nejayote* and obtain the best culture conditions for *X. campestris* xanthan production (De Monaco-Lopes et al., 2015).

It was expected that a medium without all nutritional requirements such as *nejayote*, a product with less amount of nutrients compared with controls, will be obtained. Gum produced from *nejayote* in bioreactor showed no difference, where quality properties were similar than those of commercial samples. Therefore, the present study offered an alternative in terms of obtaining this biopolymer, whose yield and production from *nejayote* are comparable with that obtained with conventional carbon sources, which meet the quality required for this product's commercialization.

Conclusions

Xanthan gum production by *X. campestris* was established using the agro-industrial residue *nejayote* as substrate, obtaining similar results compared with TGC medium in terms of biopolymer production during the first culture hours. Produced xanthan complied with humidity and viscosity parameters, but not for the particle size, based on NRF-300-PEMEX-2012 standards. Semi-industrial *nejayote* supplemented with $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (10.83 g/L) resulted the best gum production treatment. The yeast extract tested as an additive to *nejayote* had a negative effect on the xanthan production but did not reduce bacterial growth.

Production of xanthan gum can be scaled at a semi-pilot level by using this low-cost substrate, where xanthan pyruvate and acetyl values were similar than those of a commercial sample.

Methods

Xanthomonas campestris source and inoculum preparation

X. campestris strain ATCC-29497 was preserved on agar YGC (1% yeast extract, 2% D-(+)-glucose, 2% CaCO_3 , and 2% bacteriological agar) agar slant tubes, following supplier's specifications. YGC medium was prepared in distilled water, pH adjusted to 7.0,

sterilized, and placed in Petri dishes, after which they inoculated with *X. campestris* from slants and incubated for 48 h at 27 °C.

For experimental inoculum preparation, a loop from a *X. campestris* culture was transferred to a 250 mL flask containing 100 mL of YGC broth and incubated for 48 h at 27 °C at 120 rpm.

***Nejayote* source and treatments**

Industrial *nejayote* samples were obtained from Gruma, S.A. (Guadalupe, N.L., Mexico), whereas semi-industrial material was acquired from a nixtamalized corn dough production mill located in the metropolitan area of Monterrey, N.L., Mexico.

Nejayote industrial and semi-industrial samples were homogenized and filtered through an 80-mesh filter MONT INOX (Montiel Inoxidable, Monterrey, N.L., Mexico), for solids and larger insoluble material separation. After filtration, *nejayote* samples were sterilized for one hour to eliminate microorganisms present in the sample. Flasks were then prepared with 90 mL of each filtered residue and pH was adjusted to 7.0. YGC medium was used as a positive control to compare with *nejayote*-based gum production.

For the semi-industrial waste sample, different additives were evaluated as culture medium treatments to improve xanthan production, including *nejayote* with a) yeast extract (10 g/L), b) calcium carbonate (20 g/L), and c) magnesium sulfate heptahydrate (0.25 g/L) (recommended by ATCC for this strain). Treatments were sterilized, inoculated with *X. campestris* to a concentration of 1×10^8 cells/mL as a pre-inoculum, and incubated for 5 d at 27 °C and 120 rpm. Semi-industrial *nejayote* samples showing gum production were tested in 2 L flasks using 950 mL volume, completing to 1 L after *X. campestris* inoculation

(1×10^8 cells/mL), under the same incubation conditions, but increasing the stirring speed to 150 rpm and comparing the gum yield obtained with positive control. All experiments were performed in triplicate.

Xanthan gum production

Xanthan gum was recovered from samples, as previously reported (García-Ochoa et al., 2000). In brief, treatments were placed in tubes for easy handling and boiled for 20 to 30 min to eliminate remaining bacteria. Samples were then centrifuged at 10,000 rpm for 5 min at 4 °C. To separate the gum, supernatant was placed in new vials, after which isopropanol was added, using three times the volume in relation to the supernatant volume, and left at room temperature. Gum was mechanically removed using a spatula and placed on an infrared balance (AD-4715; A&D Weighing Co., CA) at 121 °C to determine dry weight and added to one liter.

Gum production among treatments was evaluated for up to 5 d culture. For industrial *nejayote* treatment, established sample amounts were collected every 8 h, whereas for semi-industrial *nejayote* samples were collected every 24 h during the culture time, gum was then separated following the protocol described above, and production values were determined and compared with those of *nejayote* without supplements.

***X. campestris* cell viability**

Cell viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl tetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO) reduction assay. For each

sample, 15 μ L of MTT (0.5 mg/mL final concentration) were added to 125 μ L of collected culture samples in flat-bottomed 96-well microplates (Corning Incorporated, Corning, NY) and incubated for 20 min, after which 20 μ L of dimethyl sulfoxide (DMSO; Sigma-Aldrich) were added to dissolve formazan crystals and optical densities (OD) read at 540 nm in a SmartSpec Spectrophotometer (Bio-Rad, Hercules, CA).

Physicochemical analysis of xanthan produced from *nejayote*

Produced gum from the industrial and semi-industrial *nejayote* was ground using a Ninja Blender model BL770A 30 (SharkNinja Operating LLC, Needham, MA). Next, 100 g of gum from industrial *nejayote* were sent to the Mexican Petroleum Institute, Services and Technology (CDMX, México) for physicochemical characteristics analysis. Resulting values were compared with those of NRF-300-PEMEX-2012 standard parameters; this Reference Standard establishes the evaluation methodology and specifications for xanthan gum to be used in oil wells drilling, completion, and maintenance fluids.

Polysaccharide-covalently bound pyruvate and acetyl content

The content of pyruvate and acetyl groups, covalently bound to xanthan, was determined because they are rheological characteristic indicators of viscosity and molecular weight, respectively (López et al., 2004; De Monaco-Lopes et al., 2015).

Pyruvate group content was measured by a colorimetric method. To completely dissolve samples, 0.5% xanthan was hydrolyzed in 0.1 M HCl for 4 h at 100 °C (Cheng et al., 2012). Next, 2.5 mL of gum suspension were placed in vials with 10% (w/v) of 0.5 M

Na₂HPO₄ and incubated at 37 °C for 1 h, after which 2 mL of 10% trichloroacetic acid were added to each vial, samples mixed and centrifuged at 2,500 rpm for 10 min, 1 mL of supernatant transferred to a new vial, and 0.5 mL of 2,4 dinitrophenylhydrazine added. This last compound was prepared by mixing 0.4 g of the reagent, adding 2 mL of concentrated sulfuric acid (with caution) and 3 mL of distilled water until dissolving, followed by adding 12 mL of ethanol and carefully shaking until homogeneity. After this, 1 mL of 0.1 M NaOH was added to each vial, and the presence of pyruvate was detected by a red precipitate; pyruvate-enriched medium was used as a positive control. Resulting mixture ODs were then read at 570 nm, using a SmartSpec Plus spectrophotometer (BioRad).

To determine acetyl content, analysis was performed as reported by McComb & McCready (1957). In brief, 200 µL of the previously hydrolyzed xanthan solution were placed in vials with 400 µL of a 1:1 mixture of 2 M hydroxylamine HCl and 3.5 M NaOH, and incubated at room temperature for 2 min, after which 200 µL of 5.65 M HCl and 200 µL of 0.37 M FeCl₂ • 6 H₂O in 0.1 M HCl were added. This solution was mixed, and the formation of a brown precipitate indicated acetyl groups presence; 0.5% commercial xanthan gum, hydrolyzed in 0.1 M HCl at 100 °C for 4 h was used as a positive control in both pyruvate and acetyl analysis. Resulting mixture ODs for pyruvate and acetyl groups determination were then read at 570 nm, using a SmartSpec Plus spectrophotometer (BioRad).

Consent for publication

All authors have read and approved the final submitted manuscript and affirm that it is not under consideration by another journal, and that no portion of this, including the abstract, has been published or posted on the internet. This manuscript contains supplementary data.

Competing Interests

The authors declare no competing interests.

Contributions

A.A, Orozco-Flores and P. Tamez-Guerra conceived the study, designed and supervised the experiments. J. O. Zúñiga-Sánchez and A. A. Orozco-Flores worked with all *nejayote* assays. J. O. Zúñiga-Sánchez, A. A. Orozco-Flores, C. I. Romo-Sáenz, M. M. Iracheta-Cárdenas and C. Rodríguez Padilla contributed to growth experiments and biomass analysis. J. O. Zúñiga-Sánchez and A. A. Orozco-Flores and R. Gomez-Flores contributed in the statistics analysis. All the authors contributed to writing the manuscript. All the authors discussed the results and commented on the manuscript and accepted the final version of the same.

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Figure legends

Figure 1. *X. campestris* cellular activity and xanthan gum production in industrial *nejayote* culture. Bars at each time represent the standard deviation. *Nj* = *nejayote*; yeast ext = yeast extract

Figure 2. *X. campestris* cellular activity and xanthan gum production in semi-industrial *nejayote* culture. A) Yield in 100 mL; B) Yield in 1 L. Bars at each time represent the standard deviation. *Nj* = *nejayote*; yeast ext = yeast extract

Figure 3. *X. campestris* cellular activity and xanthan gum production adding supplements: A) yield in 100 mL industrial *nejayote*; B) cellular activity in 100 mL industrial *nejayote*; C) yield in 100 mL semi-industrial *nejayote*; D) yield in 1 L semi-industrial *nejayote*. Bars on each column represent the standard deviation; different letter on top of each column represent significant differences (HSD, $\alpha = 0.05$).

Figure 4. *X. campestris* cellular activity adding supplements in: A) 100 mL semi-industrial *nejayote*; B) 1 L semi-industrial *nejayote*. Bars on each column represent the standard deviation; different letter on top of each column represent significant differences (HSD, $\alpha = 0.05$).

Figure 5. Pyruvate and acetyl groups covalently linked to xanthan content produced by *X. campestris* in 100 mL or 1 L semi-industrial *nejayote* after adding supplements. A) Pyruvate group; CPEM = commercial pyruvate enriched medium. A) Acetyl group. Bars on each column represent the standard deviation; different letter on top of each column represent significant differences (HSD, $\alpha = 0.05$).

Figure 6. Pyruvate and acetyl content in xanthan samples from the bioreactor using semi-industrial *nejayote*. Bars on each column represent the standard deviation; different

614 letter on top of each column represent significant differences (HSD, $\alpha = 0.05$). OD₅₇₀
615 values were comparable with xanthan commercial sample, showing pyruvate groups
616 values of 0.107 and acetyl groups values of 0.148, 0.130, and 0.127.