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Structure of xanthan gum and cell ultrastructure at different times of alkali stress

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ABSTRACT

The effect of alkali stress on the yield, viscosity, gum structure, and cell ultrastructure of xanthan gum was evaluated at the end of fermentation process of xanthan production by *Xanthomonas campestris* pv. *manihotis* 280-95. Although greater xanthan production was observed after a 24 h-alkali stress process, a lower viscosity was observed when compared to the alkali stress-free gum, regardless of the alkali stress time. However, this outcome is not conclusive as further studies on gum purification are required to remove excess sodium, verify the efficiency loss and the consequent increase in the polymer viscosity. Alkali stress altered the structure of xanthan gum from a polygon-like shape to a star-like form. At the end of the fermentation, early structural changes in the bacterium were observed. After alkali stress, marked structural differences were observed in the cells. A more vacuolated cytoplasm and discontinuities in the membrane cells evidenced the cell lysis. Xanthan was observed in the form of concentric circles instead of agglomerates as observed prior to the alkali stress.

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Introduction

Xanthan gum is a polysaccharide produced by bacterium species *Xanthomonas campestris*.[1] *Xanthomonas* cells are single straight rods, Gram-negative, 0.4–0.7 μm wide and 0.7–1.8 μm long.[2] Several biological functions, attributed to the exopolysaccharide produced by these pathogenic bacteria, include protection against adverse environmental conditions such as drying, temperature oscillations, radiation, certain chemical products, and adhesion.[3]

The production and commercialization of xanthan gum as thickener and stabilizer has progressively increased at an annual rate of 5–10%,[4] due to its better physicochemical

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properties as compared to other available polysaccharides. The most significant properties of xanthan gum include its high viscosity at low concentrations and stability for a wide range of temperatures and pH (even in the presence of salts).[5,6]

Both microorganism growth and xanthan production are influenced by several factors that include the type of bioreactor, operation mode (batch or continuous), medium composition, cultivation conditions (temperature, pH, stirrer speed, air flow rate), fermentation time, strain of the microorganisms and post-fermentation conditions (heat treatment, recovery, purification). Due to the wide application of xanthan gum and its worldwide market, several studies have been performed to optimize xanthan gum production.[4,7–13] However, the effect of this polysaccharide on the ultrastructure of cells and behavior of gum at different production stages have not yet been addressed properly, and thus the present study is relevant and may provide greater understanding on this process.

Peter et al.[14] observed accumulation of xanthan around the cells cultured on agar using transmission electron microscopy, while Contreras et al.[15] have reported plant-bacterial cell interactions during pathogenesis.

The present study aimed to evaluate the effect of alkali stress on yield, viscosity, gum structure, and cell ultrastructure, at the end of fermentation process of xanthan production by *X. campestris* pv. *manihotis* 280-95.

Material and methods

Microorganism

The lyophilized culture *X. campestris* pv. *manihotis* 280-95 was kindly gifted from the Culture Collection of the Phytopathological Bacteriology Section of the Biological Institute of Campinas (IBSBF), Campinas SP Brazil, and maintained in solid Yeast-Malt (YM) medium after activation.

Culture media

Following media were used for the culture of the bacterium: standard solid and liquid YM medium,[16] medium I[7] and nutrient medium.[17]

The standard YM medium consisted of (w/v) yeast extract 0.3%, malt extract 0.3%, peptone 0.5%, glucose 1.0%, and agar (for the solid medium) 2.5%.

The fermentation medium (Medium MP-I) comprised of (w/v) $MgSO_4 \cdot 7H_2O$ 0.02%, KH_2PO_4 0.5%, H_3BO_3 0.0006%, $(NH_4)_2SO_4$ 0.2%, $FeCl_3$ 0.00024%, $CaCl_2 \cdot 2H_2O$ 0.0002%, $ZnSO_4$ 0.0002%, citric acid 0.20% and 5% sucrose. The pH of Medium I was adjusted to 7.0 with 0.1 M HCl or 0.1 M NaOH.

Culture media were sterilized in a vertical autoclave (Phoenix VA-75) at 121 °C for 20 min. Sucrose was sterilized separately and added to the media prior to use under aseptic conditions.

Inoculum production

An aliquot of 50 mL of standard YM medium was placed in three 250 mL-Erlenmeyer flasks with vented caps. Ten standard scoops (platinum 3.0 mm diameter-scoop) of culture grown in standard solid YM for 48 h at 28 °C were added to each flask. The flasks were then incubated on an orbital shaker (New Brunswick Scientific Co. G27) at 28 °C and stirred at 250 rpm for 24 h. Cell suspensions with at least 2.6×10^9 CFU mL^{-1} [18] were used as fermentation inoculum.

Biopolymer production

After 24 h, the inoculum was transferred aseptically to a 2 L-reactor vessel containing 1.35 L of fermentation medium (Medium MP-I) and 3 drops of pure Tween 80. New Brunswick's MULTIGEN fermenter monitored to provide an airflow at 1.5 vvm, at 28.5 ± 0.5 °C and 500 rpm for 72 h was used. The pH was initially adjusted to 7.0, and a change in pH was not followed throughout 72 h.

Alkali stress

After 72 h of fermentation, 15 mL of 10 M sodium hydroxide was added to the fermentation medium until an alkali stress at pH 12 was reached. The fermented medium was sampled after 1 h, 24 h, and 48 h. The inoculum production, fermentation process, and alkali stress were performed in triplicate.

Biopolymer separation

The culture medium was centrifuged at 22,300 × *g* and 20 °C for 15 min to separate the biomass. Ethanol (92.8° GL) was added to the supernatant at a ratio of 4:1 to precipitate the biopolymer. The culture medium was stirred for 5 min, and the gum was collected on a wire mesh, placed on a glass Petri dish, frozen, and lyophilized. Then, the material was weighed and ground.

Xanthan yield

The lyophilized gum was placed in a desiccator and weighed on an analytical balance, and the gum yield was expressed by gum weight per volume of precipitated broth ($g L^{-1}$).

Viscosity

The rheological behavior of the samples was evaluated by a HAAK rheometer RS 150, using coaxial cylinder type sensor DG 41. The viscosity of the samples was determined at 25 °C. For that, 1.0% (w/v) xanthan solutions were prepared using distilled water, the mixture was stirred for 2 h, and then heated at 60 °C for 20 min.[19] The rheological analysis was performed in triplicate, using shear rate from 0.01 s^{-1} to 60 s^{-1}, with 5100 mm slot for 300 s.

Scanning electron microscopy

The structure of xanthan gum powder produced without alkali stress (W/AS) and with alkali stress (AS) at pH 12 for 1 h was assessed by scanning electron microscopy.

Gum samples were first fixed overnight in a solution containing 0.1 M sodium cacodylate buffer pH 7.4, 25% glutaraldehyde and tannic acid, and then post-fixed in osmium tetroxide. Pellets were washed thrice with sodium cacodylate

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