



Biosynthesis of Xanthan Gum by *Xanthomonas campestris* Using Cane Molasses as a Carbon Source

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ABSTRACT

The objective of the present study was to study the optimization conditions for the production of xanthan by *Xanthomonas campestris* from pre-treated sugarcane molasses. In the study, the optimization was carried out for different parameters including pH, temperature, and incubation time for the pre-treated sugarcane molasses. The age of inoculums and time of culture growth (6, 12, 18 and 24 hrs), size of inoculums (2%, 5%, 7.5% and 10%), pH (6.6, 6.8, 7.0 and 7.2) and temperature (25°C, 28°C, 30°C, 32°C and 37°C) were studied. It was observed that the xanthan production was maximal with 7.5% (v/v) inoculums, pH. 7 at 30°C for 48 hrs. The study suggested that cane molasses is an appropriate agro-industrial substrate for xanthan gum fermentations, and further scale-up study is needed for gum production in the stirred fermenter.

INTRODUCTION

Polysaccharides are important natural products usually obtained through plant sources. The manufacture of polysaccharides by the fermentation process, instead of their extraction from plant sources, is a newly developed industry (Murugesan et al. 2012, Kleinitz et al. 1989). xanthan gum is one of the polysaccharides that can be produced by culturing microorganisms belonging to the *Xanthomonas* genus (Sutherland 1998, Becker et al.1998). The natural synthesis in bacteria is a dual-stage method wherein first stage growth of the microorganism is ideal and in second stage biosynthesis of the polysaccharide takes place with considerably no growth of the microorganism (Amanullah et al.1998). The polysaccharide or gums with novel properties are in high demand in food processing and many industrial operations. FDA allowed use of xanthan gum for general use in foods e.g., in cream cheese as a thickening and stabilizing agent (Chi-Liang et al.1996, FDA 2020).

Xanthan gum is also widely used in the oil industry, the pharmaceutical industry, and a variety of other industries. (Hassler 1990). However, commercially procured xanthan is relatively expensive. The higher cost is due to the costly substrate such as glucose or sucrose and high purity required for use in food products. For example, in the synthesis of food-grade xanthan, almost half of the cost is capitalized in its downstream processing. The cost of large-scale xanthan

synthesis can be reduced by a significant amount if we use waste agricultural products as substrate (Frank & Somkuti 1979, Jean-Claude et al. 1997).

One of the best available agricultural wastes is molasses. It is a by-product of sugar production, both from sugar beet as well as from sugarcane (Pinches & Pallent 1986). Molasses is the surfeit syrup from the final stage of crystallization, from which further crystallization of sugar is profligate (Silva et al. 2009, Gumus et al. 2010). In contrast to sugar beet molasses, cane molasses differs in nitrogen levels. Also, cane molasses is a virtuous source of sucrose (almost 90%) (Cadmus et al. 1978).

Using molasses as a substrate helps to reduce the cost of xanthan production and also help address bio-waste management issue and pollution (Honart et al. 1985). In the present study, we have attempted to produce xanthan gum using cane molasses bio-waste as a carbon source. Hence, we tried to optimize the growth conditions of *Xanthomonas* strains to maximize xanthan gum production.

MATERIALS AND METHODS

Microorganism and Culture Conditions

The *Xanthomonas* strains capable of producing xanthan gums were isolated from infected cauliflower leaves. The isolated strains were screened further for xanthan gum production

and the isolate was identified based on cultural, morphological, Biochemical characteristics and molecular identification (accession number SUB9292907 16_rRNA_Xanthomonas MW741556. NCBI BLAST (<http://www.ncbi.nlm.nih.gov/Blast>). A standard strain of *Xanthomonas campestris* (NCIM 5028) bacteria procured from NCIM-Pune was also used as a Reference Culture. The isolated *X. campestris* was grown in 50 mL LB sucrose media (Sucrose: 1 g/L, Yeast Extract: 0.1g/L, Peptone: 0.5 g/L, NaCl: 0.3 g/L) for 48 h at 34°C. The isolated *X. campestris* strain was adapted to high molasses concentrations and maintained in submerged cultures on MGYE medium (Malt Extract: 0.3g, Glucose: 1g, Yeast Extract: 0.3g, Peptone: 0.5g, Water: 100mL, Agar: 2g, pH: 6.4-6.8).

Collection and Analysis of Sugarcane Molasses

The sugarcane molasses was purchased from a local source. The physico-chemical characteristics of sugar cane molasses such as color, odour, pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), acidity, total Kjeldahl nitrogen, total suspended solids (TSS), oils and grease total dissolved solids (TDS) and sulphides were conducted using the standard method (APHA 2005).

Pre-treatment of Sugarcane Molasses

The cane molasses was pre-treated by the addition of 4 g/L K₂HPO₄ to the medium to obtain better xanthan gum yield and biomass production (Murugesan et al. 2012).

Growth Adaptation of *X. campestris* in Molasses

To achieve quick growth and thereupon higher productivity, the organism was adapted to high molasses environments by successive sub-culturing (4 passages). The pure culture obtained possessed improved characteristics in terms of growth and xanthan gum manufacture (Stavros et al. 2003).

Culture Medium and Optimization

The culture Medium used was 8 % molasses pre-treated with 4 g/L K₂HPO₄. Growth parameters, viz. pH, temperature, effect of inoculum, aeration, agitation and incubation period were standardized at bench scale for the *Xanthomonas campestris* strain (isolated from cauliflower leaves) to get maximum gum production. Various parameters such as age of inoculums time of culture growth (6, 12, 18, 24 hrs), size of inoculums (2%, 5%, 7.5%, 10%), pH (6.6, 6.8, 7.0, 7.2) and temperature (25°C, 28°C, 30°C, 32°C, 37°C) and the incubation period (12, 24, 48, 72 hrs) were observed and optimized for best growth. Other factors such as aeration and agitation of culture flasks were also optimized. It was pragmatic that shaker flasks provided better aeration to the culture and agitation is required for better mass transfer.

Assay of Xanthan Gum

The final product in form of xanthan Gum was obtained in the optimized culture of *Xanthomonas campestris*. The presence of xanthan gum was determined by test method as per Indian Pharmacopoeia, 1996. Briefly, the solution was lysed with 0.1 M hydrochloric acid, the supernatant was treated with dinitrophenyl hydrazine- hydrochloric solution followed by methyl acetate and then sodium carbonate. An absorption maximum was measured at 375 nm using sodium carbonate as the compensation liquid.

Xanthan Gum Recovery

Precipitation of xanthan gum was done with isopropanol (2x volume of supernatant) to which 1 % (w/v) of potassium chloride was added. The dried biomass and xanthan gum produced were determined by drying in an oven at 105°C and 40°C, respectively and measured using Brookfield Viscometer (LABPRO, Model No. LMDV-60) at 25°C (Carignatto et al. 2011).

RESULTS AND DISCUSSION

The “xanthan gum” is produced in dual stages by *Xanthomonas campestris*. The bacterium is cultivated in Nutrient Agar and the biomass formed is used as inoculum for the subsequent stage in which the gum is formed by batch process. The biomass and xanthan gum formed were determined by drying in an oven at 105°C and 40°C. *Xanthomonas* strains capable of producing gums were isolated from infected plants (*Brassica oleracea* var. *botrytis*). Morphological and biochemical characterization of *Xanthomonas campestris* was done (Fig. 1).

To obtain a good yield of xanthan gum, various parameters such as carbon source, pH, temperature and incubation time were optimized. It was established that xanthan gum was attained in the best quantity at 30°C temperature (Fig. 2). The cell dissociation enhanced metabolite production giving high continuous yield (Psomas et al. 2007, Esgalhado et al. 1995, Garcia-Ochoa et al. 1996). A comparable observation has been made in other optimization studies wherein xanthan production was increased with a change in physical parameters (Kerdsup et al. 2011, Borges et al. 2008, Kalogiannis et al. 2003). Amongst other growth parameters, it was found that xanthan production was maximal with 7.5% (v/v) inoculums, pH.7 at 30°C at 48 hrs. (Figs. 3 and 4).

The parameters were standardized at bench scale for the effect of inoculums and age of Inoculums at different incubation time points including 6, 12, 18, 24 and 48 hrs. culture respectively. It was found that 48 hrs. were optimal for a good yield of xanthan gum. Also, the ideal size of inoculums

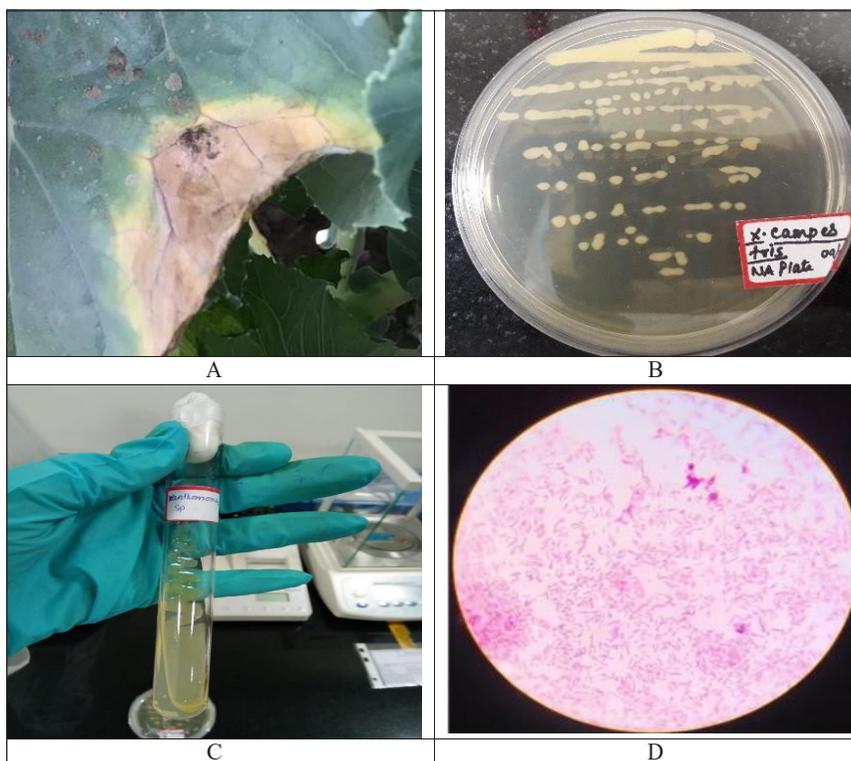


Fig. 1: A-infected Cauliflower leaf with *Xanthomonas*, B and C- isolated *Xanthomonas* strains, capable of producing gums, from infected plants (Cauliflower), D-The bacterial genus *Xanthomonas* are small, motile, Gram-negative rods, aerobic, and produce Cremish/yellow pigments and characteristically parasitize plants.

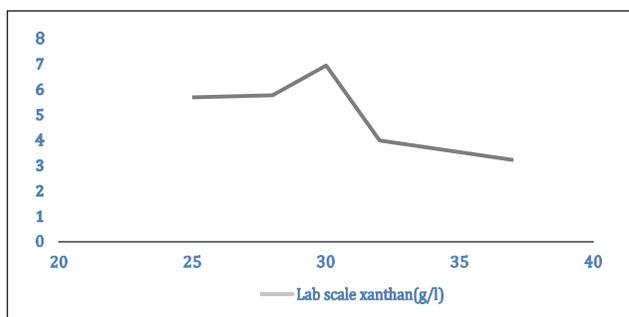


Fig. 2: Temperature variation of different quantity of xanthan production.

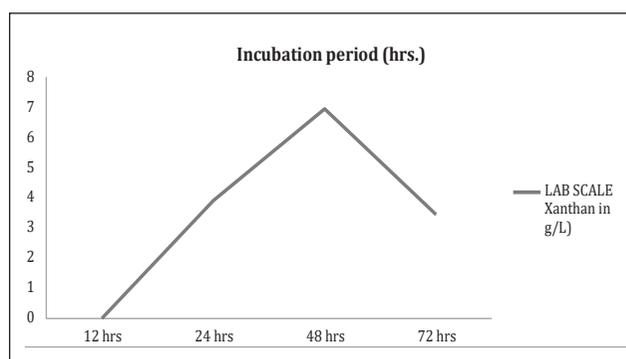


Fig. 4: Production of xanthan at different incubation time points.

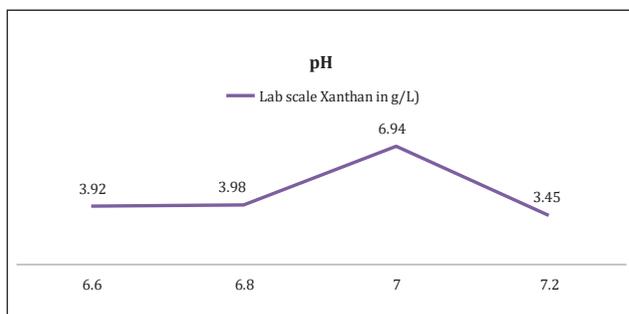


Fig. 3: Production of xanthan at different pH.

was tested to find the appropriate amount. It was observed that 12 hrs of culture as ideal age of inoculum and size of inoculums 7.5% of were best for gum yield as mentioned in Table 1. Aeration plays a vital role in determining the yield of any product in closed culture. It was observed that shaker flasks provide the right amount of aeration to *X. campestris* culture. Agitation of culture flasks is also required for better mass transfer.

This study looked into the possibility of making xanthan gum from cane molasses (8 % molasses pre-treated with 4

Table 1: Effect of inoculum.

Age of inoculum, 12 hrs culture is optimum	
Age of inoculum	Lab Scale (Xanthan gum g/L)
6 hrs	ND
12 hrs	6.94
18 hrs	4.32
24 hrs	2.34

Size of inoculum: 7.5 % is optimum	
Size of Inoculum	Lab Scale (Xanthan gum g/L)
2	3.72
5	3.96
7.5	6.94
10	2.1

g/L K_2HPO_4). In most cases, using low-cost substrates can help biological processes run more efficiently (Yoo & Harcum 1999, Stredansky & Conti 1999, Psomas et al. 2007). Glucose syrup is currently produced primarily from starchy materials. Because of the cost and availability of starch, the use of glucose syrup as a substitute for pure glucose is favored in a variety of industries, including the beverage and ethanol industries. (Kobayashi & Nakamura 2004). Cane molasses was used as a carbon source for the production of xanthan gum in this study. Indeed, this work is a first step toward increasing the efficiency of the xanthan gum manufacturing process.

The purity of xanthan gum is generally an important consideration, particularly when the product is intended for use in the food industry. The use of cane molasses in this study could help to reduce the presence of unwanted substrate ingredients in the final product (García et al. 2000, Funahashi et al. 1987). *Xanthomonas campestris* grew well on LB sucrose media and was maintained on MGY medium and cane molasses was pre-treated by addition of K_2HPO_4 to the medium to obtain better xanthan gum yield and biomass production. The strain

Xanthomonas campestris performed better in xanthan production because it had a higher maximum growth rate.

Few reports are showing different culture conditions for xanthan production using *Xanthomonas campestris* (Shu & Yang 1990). The conditions and parameters vary in each indicating that there is always a good scope of experimenting with the best conditions producing xanthan gum. This might be due to varied carbon sources and slight differences in the culture of *Xanthomonas campestris* used for inoculation.

CONCLUSION

In the current study, the *Xanthomonas* strains capable of producing gums from infected cauliflower were isolated and screened further for xanthan gum production. The potent isolated strain was used for gum production using cane molasses as a carbon source. The study concluded that xanthan production was maximum with 7.5% (v/v) inoculum, 12 hrs culture, pH.7 at 30°C for 48 hrs with the isolated strain. Further, scale-up studies of gum production in the stirred fermenter (10 L) are underway.

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