Pakistan J. Sci. Ind. Res., Vol.25, No.4, August 1982

MICROBIAL SYNTHESIS OF XANTHAN GUM BY LOCALLY ISOLATED *XANTHOMONAS CUCURBITAE**

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(Received September 20, 1981)

Biosynthesis of xanthan gum was studied using locally isolated *Xanthomonas cucurbitae* (PCSIR–52). Of all carbohydrates, such as xylose, glucose, fructose, sorbose, mannose, galactose, sucrose, maltose, lactose or starch, sucrose was found to be an ideal substrate for the production of gum. The optimum level of sugar was 3 %. The addition of corn steep liquor resulted in maximum gum formation by the culture. The pH control near neutrality by adding $CaCo_3$ (0.1%) greatly increased the formation of polysaccharide. The production of xanthan gum in 10 1 glass-stainless steel fermenter was better as compared with shake flask cultures.

INTRODUCTION

The polyanionic heteropolysaccharide called xanthan gum, newly developed by fermentation process, finds extensive use in large number of industries such as food, pharmaceuticals, oil well drilling, ceramic glazing and textile [1]. The production of xanthan gum by different species of genus *Xanthomonas* such as *X. phaseoli, X. malvacearum* and *X. carotae* has already been reported. [2, 3]. The culture of Xanthomonas campestris is widely used for the biosynthesis of xanthan gum on commercial scale [4]. The kinetic patterns indicate that multistage continuous fermentation is suitable for polysaccharide production utilizing the glucose as a sole carbon source [5]. However, certain strain variations also affect both the quality and yield of xanthan biopolymer [6]. The nutritional requirements of *X. campestris* NRRL B–1459 for the production of xanthan gum were also studied. Of all carbon sources, tested, sucrose or glucose gave optimum yield of biopolymer. Further addition of certain organic acids such as citrate, succinate, pyruvate and alpha ketoglutrate, stimulated xanthan production. The excess concentrations of these organic acids, however, inhibited the polysaccharide formation. [7,8].

The present study describes the production of xanthan gum by locally isolated culture of *Xanthomonas cucurbitae* PCSIR–52 in both shake flasks and stirred fermenter. The nutritional requirements of the culture and other parameter have also been investigated.

MATERIALS AND METHODS

The isolated cultures were maintained on YM (yeast malt extract) agar slants containing (g/1):– yeast extract, 3.0, malt extract 3.0; peptone, 5.0; glucose, 10.0; $CaCO_3$, 1.0 and agar, 20.0. The inoculum was developed in three stages using YM broth [9,10].

Xanthan gum fermentation was carried out in one liter cotton wool plugged conical flasks. The flasks were rotated at 150 rpm on a rotary shaker. The basal medium consisting of (g/1) glucose or other carbon source, 30.0; K_2HPO_4, 8.0; $MgSO_4.7H_2O$, 0.2; $(NH_4)_2HPO_4$, 1.5; was sterilized at 121° for 15 min. The sugar solution was sterilized separately and added aseptically before inoculation. The pH was adjusted at 7.0 ± 0.2 with 2.5 N NaOH or H_2SO_4. The shake flasks after inoculation were incubated at 28± 1° for 72 hr. The production of xanthan gum was also carried out in 10 liter glass-stainless steel baffled fermenter, designed and fabricated in PCSIR Laboratories workshop using QVF pipe with stainless steel plates at both ends provided with agitation, aeration and cooling systems. The rates of agitation and aeration were 200 rpm and 0.5–1.0 1/1/min. respectively. The sterilized sugar and salt solutios were transferred aseptically to steam sterilized

*This work has been conducted under the Pakistan Science Foundation grant No.PSF/RES/P–CSIR/Chem (103).

fermenter. The air was supplied after passing through sterilized glass wool tubes. The culture medium was inoculated at the rate of 10 % v/v by 24 hr. old vegetative inoculum developed in shake flasks.

The viscosity of fermented broth was determined by Ostwald Viscometer at 30° after appropriate dilution. The residual sugar was estimated by the method of Lo and Garceau *et al.* [11]. The xanthan gum was determined by the method of Jeans *et al* [12].

RESULTS

Screening of Organisms. The isolated four cultures of *Xanthomonas sp.* were selected for the biosynthesis of Xanthan gum in shake flasks (Table 1). The shake flasks cultures were analysed 68 hr after inoculation with 24-hr-old seed cultures. Of all strains, however, *X. cucurbitae* (PCSIR–52) produced maximum xanthan biopolymer (8.1 g/l). The viscosity of fermentation broth was 1.55×10^{-3} cp. The production of gum by other isolates of the genus *Xanthomonas* was also satisfactory. *Xanthomonas cucurbitae* (PCSIR–52), however, was selected for subsequent studies in shake flasks and stirred fermenters.

Nutritional Studies of X. cucurbite *(PCSIR–52) in shake Flasks. (a) Effect of Carbohydrates:* The data of table 2 indicates the effect of various carbohydrates such as Xylose, glucose, fructose, sorbose, mannose, galactose, sucrose, maltose, lactose or starch, on the biosynthesis of Xanthan gum by the organism in shake flasks. The concentration of each carbohydrate was kept at 3 % (w/v). The polysaccharide formation was maximum in the presence of sucrose, i.e., 15.0 g/l, and decreased in the order of lactose, galactose, sorbose, maltose, glucose, mannose, starch, fructose and xylose. The production of polysaccharide was better in the presence of disaccharides as compared with monosaccharides. The gum formation was quite low in the presence of xylose. For subsequent experiments, therefore, sucrose was used throughout for the production of xanthan gum.

(b) Effect of Corn-steep Liquor and Cabbage extract: Corn-steep liquor (CSL), a by-product of starch industry, is a good source of nitrogenous compounds and other growth factors. The CSL obtained from Rafhan Maize Products was concentrated in a steam-jacketted vessel. The moisture level of corn-steep liquor was about 30 %. Effect of the addition of CSL (0–9 %w/v) on the production of Xanthan gum by *X. cucurbitae* (PCSIR–52) was investigated (Fig.1). The synthesis of exopolysaccharide was stimulated by adding corn-steep liquor. The optimum level of CSL was 0.6% w/v and the amount of gum formation was

Table 1. Screening of xanthomonas sp. capable of producing xanthan gum.

Isolates	Viscosity* cp x 10^{-3}	Xanthan g/l
X. campestris (PCSIR–51)	1.03	6.0
X. cucurbitae (PCSIR–52)	1.55	8.1
X. richinicola PCSIR–53)	1.2	7.1
X. citri (PCSIR–54)	1.1	6.2

*Viscosity at 30°

Table – 2. Effect of carbohydrate on the biosynthesis of xanthan gum by *X. cucurbitae* (PCSIR–52)

Carbohydrates	Viscosity* cp x 10^{-3}		Xanthan g/l
Xylose	1.06		5.0
Fructose	2.10		9.7
Glucose	2.20		9.8
Sorbose	3.11		10.2
Mannose	2.30		9.6
Galactose	3.27		9.3
Sucrose	5.59		15.0
Maltose	3.09		9.3
Lactose	4.51	14.1	14.1
Starch	2.0		8.7

*Viscosity at 30°.

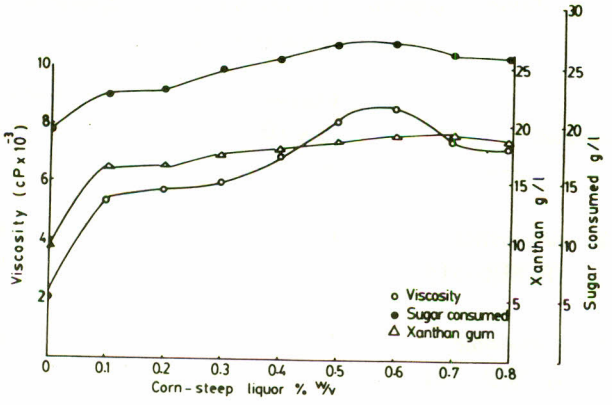

Fig. 1. Effect of corn-steep liquor on the production of Xanthan gum by *X. cucurbitae* (PCSIR-52) in shake flasks.

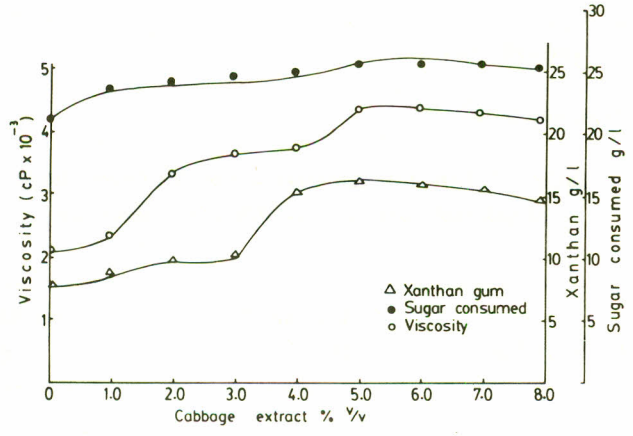

Fig. 2. Effect of cabbage extract on Xanthan formation by *X. cucurbitae* (PCSIR-52) in shake flasks.

19.3 g/l. The viscosity of fermentation broth was $9x10^{-3}$ cp. The exact mechanism of simulatory effect of CSL, however, needs further studies.

Effect of cabbage extract on the production of xanthan gum, was also investigaaed. The cabbage extract was prepared by refluxing 100 g of crushed cabbage leaves in one liter tap water. The extract was centrifuged at 3,000 rpm. for 15 min. and the clear supernatant was added v/v to the fermentation medium (Fig.2). The gum formation was maximum when 5.0 ml of the extract was added to the fermentation medium in shake flasks. The amount of polysaccharide was 16.0 g/l. Further increase in the concentration of cabbage extract resulted in decrease of both gum formation and viscosity of culture broth.

(c) Effect of Calcium Carbonate: The influence of pH control by adding calcium carbontate (0–2.5 g/l) to the fermentation medium was also investigated (Fig.3). The pH of the fermented broth remained at about 6.0 during the fermentation. The production of xanthan gum by the organism was increased by the addition of $CaCO_3$. The optimum level of the salt was 1.0 g/l and the amount of gum produced was 17.2 g/l. Further increase in the concentration of $CaCO_3$ decreased the gum formation. For pH control in the fermented broth, therefore, $CaCO_3$ was added at the level of 1.0 g/l in further investigations.

(d) Effect of Thiourea: The data of Fig.4 indicates the effect of the addition of thiourea in shake flasks experiments on gum synthesis. The production of xanthan polysaccharide by *X. cucurbitae* (PCSIR–52) was maximum, i.e., 16.6 g/l in the presence of 0.3 % (w/v) thiourea (Fig.4). The viscosity of fermented broth was doubled than that of control culture, i.e., $2.5 - 4.2 \times 10^{-3}$ cp respectively. The formation of gum, however, was decreased by futher increasing the concentration of thiourea. The exact machanism of stimulatory effect of thiourea on gum synthesis is not clear and needs further studies.

Biosynthesis of Xanthan Gum in 10 Liter Glass-Stainless Steel Stirred Fermenter: The scale up studies of xanthan gum fermentation was also carried out in 10 l glass-Stainless steel fermenter. The fermentation medium (5 liter)

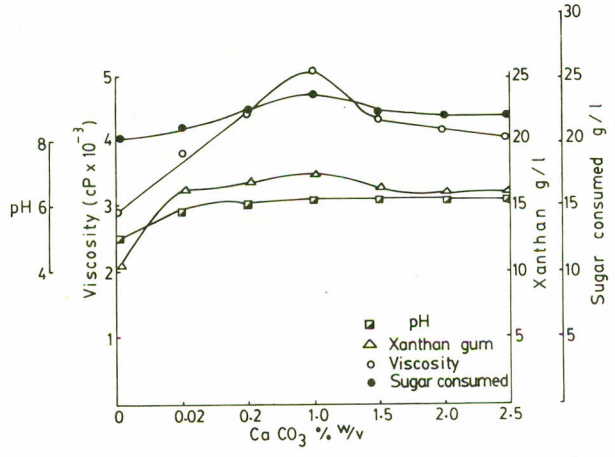

Fig. 3. Effect of calcuim carbonate on Xanthan gum formation by *X. cucurbitae* (PCSIR-52) in shake flasks.

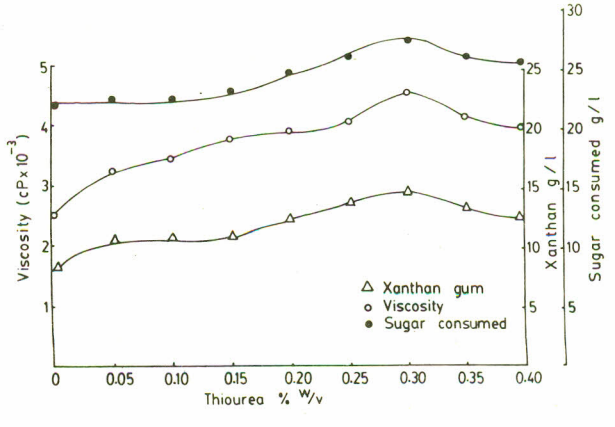

Fig. 4. Effect of thiourea on the production of Xanthan gum by *X. cucurbitae* (PCSIR-52) in shake flasks.

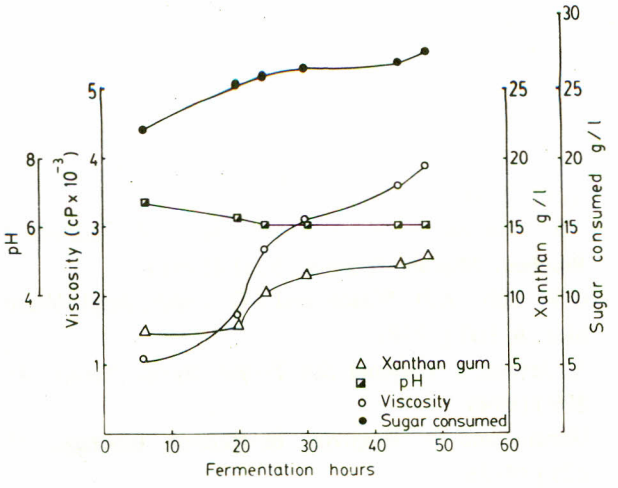

Fig. 5. Effect of glucose on the biosynthesis of Xanthan gum by *X. cucurbitae* (PCSIR-52) in 10-L glass-stainless steel fermenter.

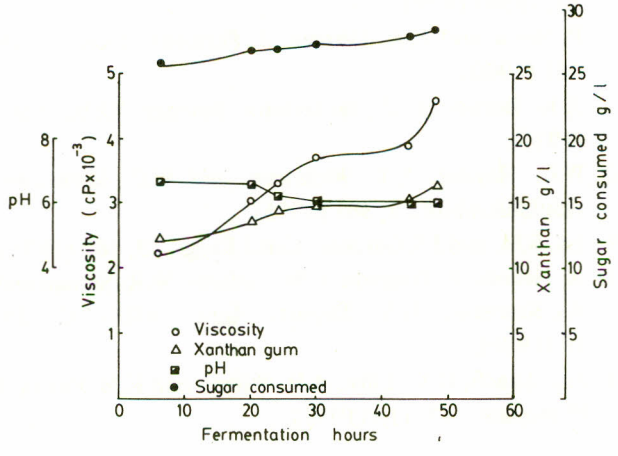

Fig. 6. Effect of lactose on the biosynthesis of Xanthan gum by *X. cucurbitae* (PCSIR-52) in 10-L glass-stainless steel fermenter.

after sterilization, was aseptically transferred to steam sterilized fermenter. The rate of agitation and aeration were 220 rpm and 1.0 1/1/min respectively. The size of 24-hr-old inoculum, developed in shake flasks, was 10 % v/v. The substrates such as glucose, sucrose or lactose were evaluated for gum formation in the fermenter (Figs. 5–7). The pH near neutral was kept by adding sterilized calcium carbonate (1.0 g/l). The rate of fermentation in the stirred vessel was greater than that of shake flasks. That is, gum production reached maximum 48 hr. after inoculation in the fermenter than in shake flasks, i.e., 72 hr. The amount of gum produced by *X. cucurbitae* (PCSIR–52) was maximum in the presence of sucrose (18.0 g/l) than lactose (16.21 g/l) or glucose (13.0 g/l).

Further experiment was also performed in the fermenter containing sucrose, $CaCO_3$, corn-steep liquor and

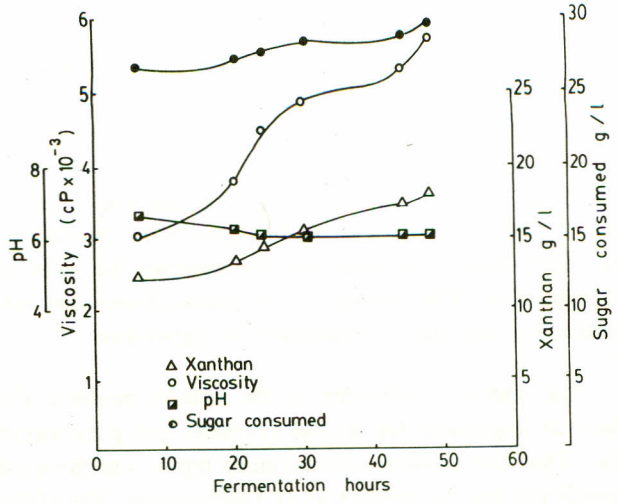

Fig. 7. Effect of sucrose on the production of Xanthan gum by *X. cucurbitae* (PCSIR-52) in 10-L glass-stainless steel fermanter.

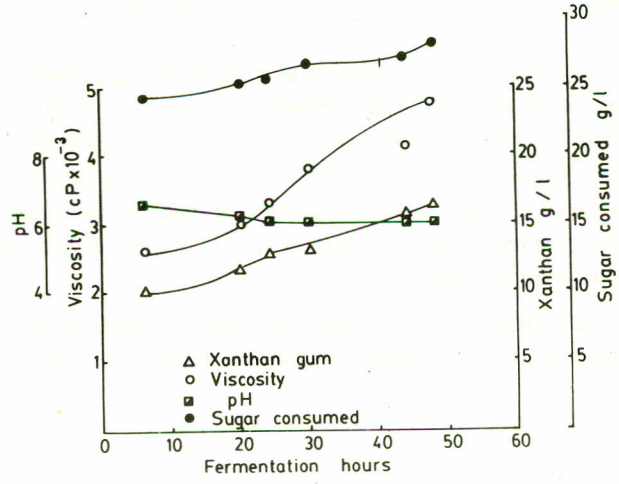

Fig. 8. Combined effect of thiourea and corn-steep liquor on Xanthan biosyntheses by *X. cucurbitae* (PCSIR-52) in 10-L glass-stainless steel fermenter.

thiourea (Fig.8). The production of xanthan gum was slightly affected as compared with surcose, but equal to lactose. The results, however, were greater than the shake flasks.

DISCUSSION

Xanthan gum fermentation by locally isolated cultures of *Xanthomonas species* was studied by submerged fermentation in both shake flasks and in a stirred fermentor. Out of four cultures of *Xanthomonas sp.* isolated from infected plants, *X.cucurbitae* (PCSIR–52) produced maximum biopolymer. The sugars such as xylose, glucose, fructose, sorbose, mannose, galactose, sucrose, maltose, lactose or starch

were evaluated from gum synthesis. Of all sugars tested for gum synthesis sucrose gave optimum yield of Xanthan gum. This observation is in accordance with *Xanthomonas campestris* NRRL B–1459 as reported by Leach *et al* [13] and later by Souw and Demaina [7]. The concentration of sugar in the basal medium is also very critical. The optimum level of sugar was found to be 3% w/v. Further increase in the concentration of sugar did not improve the yield of Xanthan gum. The viscosity of fermented broth was responsible for low sugar consumption at higher level.

The source of nitrogen in the culture medium also plays an important role in cell synthesis and gum formation. Thus the addition of corn-steep liquor was the most stimulatory for the production of Xanthan gum. This effect may be availability of growth factors alongwith nitrogenous compounds. Effect of adding cabbage extract on gum formation was not encouraging. Addition of thiourea to the basal medium did not show any significant increase in the viscosity of fermented broth and gum formation.

pH of the basal medium was lowered during fermentation and it resulted in the decrease of gum yield. Addition of $CaCO_3$ near neutrality, greatly enhanced the biosynthesis of Xanthan gum. The optimum of $CaCO_3$ was found to be about 1.0 g/l. Further increase in calcium carbonate concentration, however, showed no significant effect on biopolymer synthesis.

Attempts were also made to scale up Xanthan gum production in the stirred fermentor using 3 % sucrose, lactose or glucose as carbon source. The three sugars were equally effective in the polysaccharide formation.

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