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A COMPARATIVE STUDY ON BIOSYNTHESIS OF XANTHAN GUM USING THREE DIFFERENT *XANTHOMONAS* STRAINS ISOLATED FROM DISEASED PLANTS

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ABSTRACT

Naturally occurring bacterium Xanthomonas strains like X.campestris,X. malvacearum and X.axonopodis were isolated from diseased plants and identified. They were used for xanthan gum production by fermentation with different carbon and nitrogen sources. The effect of pH and temperature were also determined during the production of xanthan gum. This extracted polymer was then purified with alcohol, dried and milled. The viscosity of the xanthan gum produced was found. The biomass and yield of xanthan gum were determined.

KEYWORDS : **Xanthan gum, Fermentation, *x.campestris*, Temperature, viscosity**

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INTRODUCTION

Xanthan gum is a natural polysaccharide and an important industrial biopolymer. It is produced by a pure culture fermentation of a carbohydrates by naturally occuring *Xanthomonas* strains like *Xanthomonas campestris, xanthomonas malvacearum and xanthomonas axonopodis*. This polymer is one of the major microbial polysaccharides actually employed in many industrial processes due to its rheological behavior and pseudoplastic in nature. Xanthan is a white to cream color free flowing powder soluble in both hot and cold water to give viscous solution at low concentration. This makes xanthan very useful in suspending, stabilizing, thickening and emulsifying agent for food, cosmetics, pharmaceuticals and oil recovery [1]. The structure of xanthan gum consists of repeating polysaccharide units D- glucose, D-mannosyl, D-glucuronyl acid residues in a molar ratio of 2:2:1 and variable proportional of O-acetyl and pyruvyl residues. Xanthan gum is an acidic polymer made of pentasaccharide subunits forming a cellulose backbone with trisaccharide side chains composed of mannose (β 1,4) and glucuronic acid (β 1,2); mannose is attached to alternate glucose residues in the backbone by α 1,3 linkages. The mannose reside nearest the main chain can carry a pyruvate group between C_4 and C_6. [2]. Many variables such as the composition of the culture medium, temperature, pH and oxygen transfer rate affect the production of xanthan. [3,4,5]. Most commercial production methods for xanthan gum use glucose or invert sugars and most industries prefer batch process to continuous process [6]. Other substrates such as hydrolyzed rice, barley, corn, flour, acid whey, molasses, coconut juice, sugarcane etc., were used for the production [7, 5]. *Xanthomonas* consists of gram negative rod shaped polar – flagellated bacteria whose members commonly occur as serious plant pathogens. *X.axonopodis* causes citrus canker which imparts heavy economic losses on citrus industries. The pathogen expresses itself infected citrus plants as brown, raised lesions on leaves and fruits with oily water soaked and necrotic margins [8]. *X. malvacearum* is a phytopathogenic bacterium that causes bacterial blight of cotton. The bacteria can attack hosts during all growth stages [9]. This study represents isolation characterization of three different *xanthomonas* from affected plantlets. The objective of this study was to use different carbon sources in a fermentation medium for the production of xanthan gum and compare its yield with physical parameters under laboratory conditions in batch fermentation.

MATERIALS AND METHODS

Xanthomonas axonopodis was isolated from citrus canker fruits and leaves. They were excised using a sterile razor in the laminar and were surface sterilized using 70% alcohol. Lesions were tweezed by sterile forceps and left aside for 10 mins. A loopful of tweezed bacteria was taken and streaked on the yeast extract peptone media. *Xanthomonas campestris* was isolated from blackrot of cabbage. The excised portions were washed with 0.5% sodium hypochloride solution. The macerated portions were maintained at yeast malt extract agar plate. For *xanthomonas malvacearum,* the infected leaf portion were excised from the bacterial blight of cotton (Gossypium hirsutum) .Infected leaf portions were washed with 0.5% sodium hypochloride and macerated portion was suspended in distilled water and spread on Yeast malt extact agar plate. Yellow and small mucoid colonies were formed and examined microscopically for the morphological characterization. Viable mucoid colonies were maintained on yeast agar slant and sub culture was made after every two weeks and stored at refrigerator.

***Inoculum development**:*

Actively growing cells from a freshly prepared yeast malt extract agar slants were inoculated into 100 ml yeast extract malt liquid

medium containing (g\l) of malt extract 3.0, glucose 20.0 at pH 7. The seed culture developed was used for both shake flasks and fermenter studies. Seed culture development was carried out in1.5 liter stainless steel fermenter for the production of xanthan gum.

Fermentation Technique:

Xanthan gum production was carried out in 1 liter conical flask containing 100ml fermentation medium. The substrate used was glucose 40 g/l, $KH_2PO_4$2.1g/l, $Mgcl_2$ 2.86g/l, $Na_2SO_4$0.50g/l,.$H_3BO_3$0.006 g/l, ZnO 0.0006 g /l, $Fecl_3$6.H_2O 0.020 g/l and $CaCO_3$ 0.020g/l. Corn oil was used as an antifoaming agent. The optimum temperature maintained was 30^0c to 34^0c and pH was maintained from 5 to 7 for *X.malvacearum* (Dermain AL, 2000)[10]. For *X.campestris* and *X.axonopodis sp*. the temperature and pH were found to be 28^0 C and 5-7. Nutritional studies of the isolated cultures were carried out in shake flasks before scale up production in stirred fermentation by batch process.

Effect of carbon sources and nitrogen sources

To the chemically defined medium in replacement of glucose as carbon source 0.5% of fructose, sucrose and galactose were used. 2% commercial available nitrogen sources as peptone, beef extract, tryptone were used to determine xanthan gum production was measured at 30^0c and 34^0c for 96 hours.

Recovery and determination of xanthan gum

The main steps for the recovery process were deactivation, removal of microbial cells, precipitation of the biopolymer, dewatering, drying and milling. The crude xanthan gum was isolated from the fermentation broth and bacterial cells can be removed by centrifugation with alcohols such as methanol/ isoproponal. This was further precipitated by adding potassium chloride and 95% ethanol. A gelatinous flocculants at low density was separated by centrifugation. The residue was transferred to pre-weighed aluminum foil cups and dried for 18 hours in a hot air oven at 60^0c [11].

Estimation of biomass

Growth of the organism was determined by measuring the dry weight of the washed mass. The bacterial cells containing broth was centrifuged at 5000 rpm for 15 minutes. The pellet was washed twice with deionized water and dried at 80^0c-100^0c.Viscosity of the sample was measured by using vibro-viscometer [12].

RESULTS AND DISCUSSION

To produce xanthan gum, xanthan strain needs several nutrients include micronutrients (potassium, iron and calcium salts) and macronutrients (carbon and nitrogen sources). Sucrose, fructose and galactose were used as carbon sources.2-4% of carbon concentration was preferred for xanthan production. The best carbon source was found to be sucrose for production of xanthan gum at optimum temperature . Peptone was the best nitrogen source at the concentration of 15mM (Figure 1). *X.malvacearum* showed a maximum yield (0.35g/100ml) at optimum temperature 34^0c using sucrose as carbon source and peptone as nitrogen source. Galactose showed minimum production of xanthan gum at both temperature of 30^0c and 34^0c (0.05g/100ml). Xanthan gum production was rapidly declined from 0.25g/100ml to 0.05g/100ml using tryptone as nitrogen source. This was due to changes in temperature and nutrient sources. To determine optimal production of xanthan gum by *X.malvacearum* using pH of 5 and 7 in fermentation medium with different carbon and nitrogen sources were studied. Maximum yield of xanthan (0.25g/100ml) using sucrose as carbon source at pH 5 when compared to different sugars even at pH 7.The optimum production of xanthan gum production was observed at pH 7only when peptone and tryptone were used as nitrogen source(Figure

2). The yield of xanthan from the fermentation medium was gradually declined by changing pH from 5to7 using different sugars, but rapidly increased in the production of xanthan gum at pH7 with two different nitrogen sources except beef extract. The pH of the fermentation broth was reduced fast because of acidogenic nature of xanthan gum and byproducts which affect the conversion of sugars to oligosaccharides. Therefore, the maintenance of pH was essential for maximum yield of xanthan gum. Figure 3&4 showed change in biomass of *xanthomonas campesteris* using different carbon and nitrogen sources. The biomass was gradually increased during fermentation period due to increase in its growth. In a study by Rosalam and England (2006)[5] it was noted that increased biomass production yield can be obtained at high nitrogen sources. The maximum yield of biomass (0.40mg/ml) was observed in beef extract as nitrogen source at incubation period of 72 hours. Thus incubation temperature is an important critical factor in biomass production. The maximum yield of xanthan gum (12.5g/kg) was observed in *x.campestris* when compared with other xanthomonas strains (Figure 5). Among the three strains *x.axonopodis* showed minimum yield (3.4g/kg) of xanthan gum production whose viscosity of 6.5 cP. *X.malvacearum* had a viscosity of 6.5cP in the yield of 8.6g/kg. The viscosity of xanthan gum is affected by changes in pH. A slightly rise in pH above 7, xanthan gum is gradually acetylated. Thus, x.campestris showed maximum yield of xanthan gum and its high viscosity using sucrose as carbon source and tryptone as nitrogen source.

CONCLUSION

Xanthan gum is a predominant component of bacterial slime. Efficient conversion of carbon sources to the desired polysaccharide production requires a high carbon to nitrogen. The effect of pH and temperature for xanthan gum using three different *xanthomonas* were determined. Nutritional studies were analyzed using different carbon and nitrogen sources. Optimization of xanthan production was carried out at specific pH, carbon source (sucrose) and viscosity.

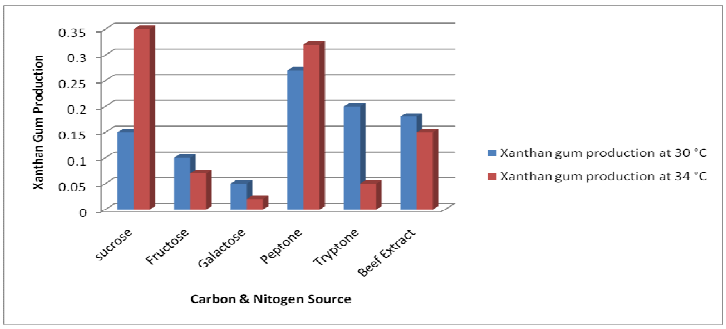

Figure 1
Effect of Temperature on xanthan gum production using different carbon and nitrogen sources in x.malvacearum

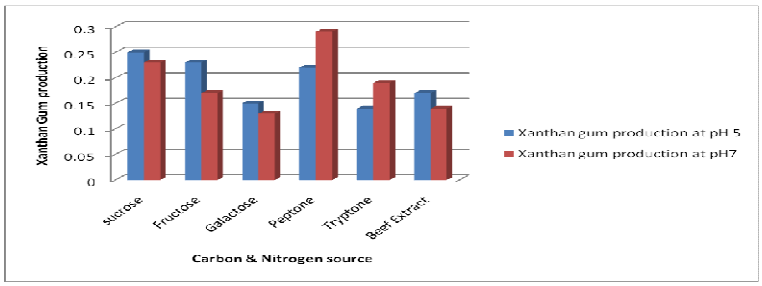

Figure 2

Effect of pH on different carbon and nitrogen sources for xanthan production using x.malvacearum

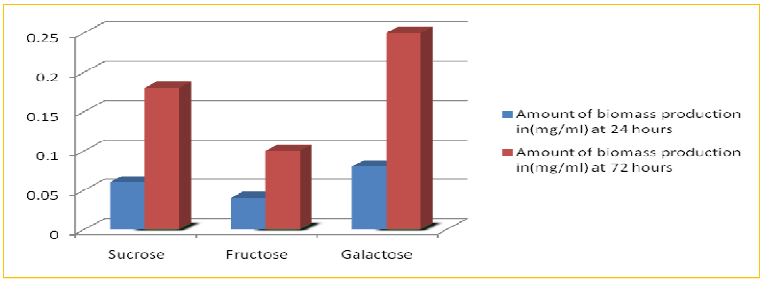

Figure 3

Effect of Carbon sources for Xanthan Production.

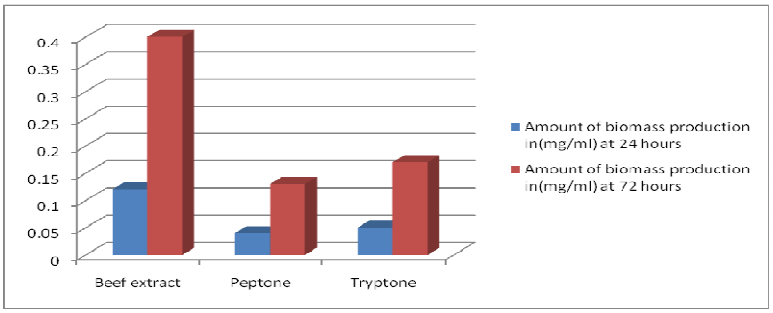

Figure 4

Effect of Nitrogen sources for Xanthan Production.

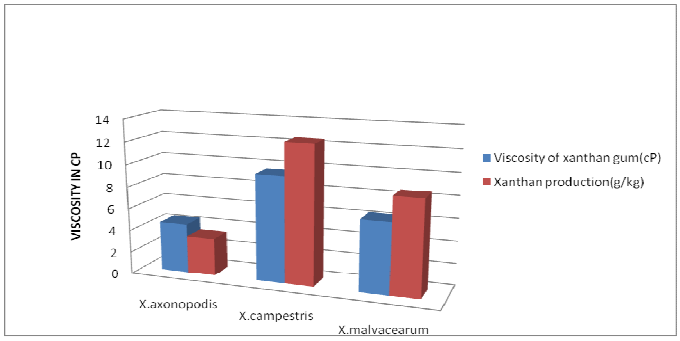

Figure 5

Production of Xanthan Gum using three different strains and its Viscosity

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