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Relationship of Biomass and Xanthan Gum Production by *Xanthomonas campestris*: Optimization of Parameters

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Authors' contributions

The present work is result of the efforts of the both the authors. Both of the authors have contributed substantially, the senior author MMVB designed the experimental and wrote the draft of the manuscript. The junior author SC performed the experiments and all analytical methods used in the study. Both the authors read and approved the final draft.

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Original Research Article

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ABSTRACT

Aims: To determine the relationship of biomass and xanthan gum production by *Xanthomonas campestris* and to optimize the environmental and nutritional parameters for their production.
Study Design: *X. campestris* strains were screened for the production of xanthan gum. Thirty eight strains isolated from various districts in Maharashtra were included for this study. Of these thirty eight strains, one is found to be efficient producer of xanthan gum and slight variation among the strains was recorded. The effect of various parameters viz., time, pH, temperature, carbon source and nitrogen source was studied.
Place and Duration of Study: Research laboratory, Department of Biotechnology, Yeshwant Mahavidyalaya, Nanded, India.
Methodology: Isolation of desired strain was carried out by using various morphological and biochemical tests. Analysis of functional group of synthesized xanthan gum was determined by FT-IR spectroscopy.

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Results: It was found that the maximum production of biomass and xanthan gum was recorded at 96 hrs. The optimum pH was 6.5 for biomass and pH 6 for xanthan gum production whereas 35 ℃ was optimum temperature for both *biomass* and xanthan gum production. Sucrose was most preferred carbon source for xanthan gum and biomass production while yeast extract was best nitrogen source for xanthan gum production whereas peptone was most suitable nitrogen source for optimum production of biomass.
Conclusion: the optimized environmental and nutritional parameters obtained from this study will further help to maximize the production of Xanthan gum by *X. campestris*.

Keywords: Xanthomonas campestris; xanthan gum; biomass; surface fermentation; FT-IR spectroscopy.

1. INTRODUCTION

The plant pathogenic bacterium *Xanthomonas campestris* produced an anionic polysaccharide named Xanthan Gum [1]. In late 1950s American research team discovered Xanthan gum, when conducting extensive search for useful biopolymers [2] and it found to be widely used in food products. Xanthan Gum consists of a linear 1, 4-linked β-D-glucose backbone as a main chain, substituted on every two unit with a charged tri-saccharide side chain. Other side chain is composed by a D-glucuronic acid unit linked between two D-mannose units [3]. The gum exhibits many advantages as a thickener, stabilizer, gelling agent and suspending agent, as creams, artificial juices, sauces for salads, meat, chicken or fish, as well as for syrups and coverings for ice creams and desserts as it has high degree of stability and solubility [4]. Xanthan Gum is combined with galactomannans for the use as a gelling agent due to its weak gel structures [5]. Xanthan is useful in many industries as its nature as a non-toxic, it is also tested that Xanthan does not inhibit growth. Xanthan is more beneficial as it is non-sensitizing and does not cause skin or eye irritation [6].

Many studies pertaining to production of xanthan gum were carried out with strains from cultures collection. The production of xanthan is influenced by various media components and culture conditions. So, in this study the result of the wild strains isolated from various districts of Maharashtra were screened for production of xanthan gum and the production of xanthan gum and the most efficient strain was further used in the study and the results are reported in this paper.

2. MATERIALS AND METHODS

2.1 Isolation of *Xanthomonas* Species

Thirty eight strains of *X. campestris* isolated from *Citrus lemona* from different locations and districts of Maharashtra were used in this study. All the strains were isolated from infected plant parts during mid-rainy season following standard method. All these strains were purified and maintain on YDC (Yeast extract -1 gm, D-glucose -2 gm, Calcium carbonate -2 gm) slants.

2.2 Morphological Characteristics

Morphological characteristics were recorded for all these strains like colony characters, Gram staining, Cell morphology, Cell motility, etc [7].

2.3 Biochemical Test

Further characteristics of the isolated strain were examined by using the biochemical test described by Goszczynska et al. [8], Aesculin test, Starch hydrolysis, Tween 80 lipolysis, H2S production, Urease production, Milk proteolysis, Gelatin liquefaction, and Oxidase test.

2.4 Production Media for Xanthan gum

The media used for the production of Xanthan gum contained, D-Glucose 20 gm, Yeast extract 3 gm, K2HPO4 2 gm, MgSO4.7H2O 0.1 gm, Distilled water 1000 ml. Fifty ml of the medium was taken in each 100 ml Erlenmeyer flask and used for further study.

2.5 Optimization of Cultural Parameters for Biomass and Xanthan Gum Production

2.5.1 Effect of incubation time on biomass and xanthan gum production

The optimum time for biomass and xanthan gum production was determined by using different time intervals as 1st day, 2nd day, 3rd day, 4th day and 5th day. The experiments were conducted using flasks (250 ml) containing 50 ml xanthan gum production medium. These flasks were

autoclaved at 15 lbs for 20 min. Then culture of *X. campestris* (*Xan 22*) was inoculated in each flask. All the flasks were incubated and flasks were drawn at different time of incubation as previously mentioned by keeping all other conditions constant for carrying out surface fermentation.

2.5.2 Effect of temperature on biomass and xanthan gum production

For the determination of optimum temperature for biomass and xanthan gum production, flasks (250 ml) containing 50 ml of xanthan gum production medium were autoclaved at 15 lbs for 20 min. Then the flasks were inoculated with the culture of strain *Xan 22* and incubated at different temperature ranges from 25℃, 30℃, 35℃, 40℃, 45℃ at static condition by keeping all other process parameters constant [9].

2.5.3 Effect of pH on biomass and xanthan gum production

To determine the optimum pH for biomass and xanthan gum production, different pH media of 6, 6.5, 7, 7.5, 8, and 8.5 were used. flasks of 250 ml containing 50 ml xanthan gum production media were adjusted to pH of the medium by using 1 N HCL or 1 N NaOH. The flasks were autoclaved at 15 lbs for 20 min and were inoculated with the culture of *Xan 22* in each flask and the flasks were incubated for surface fermentation by keeping other conditions constant. Thus the effect of different pH on production of xanthan and biomass were determined [9].

2.5.4 Effect of carbon sources on biomass and xanthan gum production

Flasks of 250 ml containing 50 ml xanthan gum production medium with different carbon sources such as starch, sucrose, lactose, and maltose instead of glucose with same concentration as 2% w/v were used to study the effect of carbon sources. The flasks were autoclaved at 15 lbs for 20 min. and then were inoculated with culture of *Xan 22* and these flasks were incubated by maintaining other conditions constant [10].

2.5.5 Effect of nitrogen sources on biomass and xanthan gum production

The effect of nitrogen sources on production of biomass and xanthan gum was studied keeping all other process parameters constant. Different nitrogen sources such as peptone, Ammonium sulphate, Ammonium nitrate, Potassium nitrate were used in the production medium of xanthan gum instead of yeast extract at the same concentration of 0.3 % w/v. All the flasks were autoclaved at 15 lbs for 20 min. Then were inoculated with the culture of *Xan 22* and all flasks were incubated at static condition [10].

2.6 Extraction of Biomass

Biomass was recorded as the dry weight of washed mass of cell. After the experiments of optimization parameter for biomass and xanthan gum production, all flasks were centrifuged for extraction of biomass and xanthan gum. The medium was centrifuged at 10,000 rpm for 15 min. and two fractions were formed in the centrifuge tube. The biomass was deposited as a pellet and xanthan gum was present in supernatant. Thus the biomass pellet was suspended in deionized water for washing. Further it was recentrifuged at 4000 rpm for 10 min. to precipitate the biomass. The biomass was deposited at the bottom of centrifuge tube and was collected in pre-weighed plate with aluminum paper and dried in the oven at 60℃ for two hours and weighed to determine the dry mass per liter medium.

2.7 Extraction of Xanthan Gum

The supernatant collected from extraction of biomass was mixed with 2 to 3 volumes of ethanol with continuous shaking to precipitate the xanthan gum. The obtained precipitate was separated by centrifugation at 6000 rpm for 15 minutes. The collected residue was transferred in preweighed micro-centrifuge tube. This tube was kept in hot air oven for drying at 60℃ for 20 hours. The micro-centrifuge tube was cooled at room temperature then dry weight was determined. The obtained dry weight gave the concentration of xanthan gum by calculating the dry weight of xanthan gum per liter medium.

2.8 Analytical Method

Analysis of functional group of synthesized xanthan gum was performed by using FT-IR Shimadzu 8400S spectroscope. For FT-IR spectra dried powder of xanthan gum was used. This dried powder of xanthan gum was incorporated into KBr and pressed into pellet under pressure. The transmittance mode used during analysis was 4000 to 400 cm-1.

3. RESULTS AND DISCUSSION

X. campestris was isolated from infected citrus leaves for the study of xanthan gum production. Xanthan Gum and biomass production are affected by the various biochemical parameters like, time, pH, temperature and other media components like C – Sources (starch, sucrose, glucose, lactose, and maltose) and N-Sources (yeast extract, peptone, ammonium sulphate, ammonium nitrate, potassium nitrate).

3.1 Optimization of Cultural Parameters for Biomass and Xanthan Gum Production

3.1.1 Effect of different incubation time on biomass and xanthan gum production

Experiments were conducted to optimize the incubation time for biomass as well as xanthan gum production, different time intervals were selected as 1st day, 2nd day, 3rd day, 4th day and 5th day. The production of biomass and xanthan gum was found to increase continuously from 1st day to 4th day of incubation but as incubation time extended from 4th day to 5th day there was decrease in production of both as biomass and xanthan gum. Hence optimum time is 96 hrs. for biomass production and xanthan production and maximum biomass production was 24.18 g/L whereas maximum xanthan yield was 15.21 g/L (Fig. 1). Earlier studies have shown that the maximum xanthan production was after 48 hours and increased with increase in time [6,9].

3.1.2 Effect of temperature time on biomass and xanthan gum production

The effect of temperature on xanthan production in temperature ranges from 25℃, 30℃, 35℃, 40℃, 45℃ was studied. It was found that as temperature increased from 25℃ to 35℃, the production of biomass and xanthan gum also increased but further increase in temperature lead to reduction in production of both biomass as well as xanthan gum. It was clear that the optimum temperature for biomass and xanthan gum production was 35℃ and maximum production of biomass was found to be 23.29 g/L and the maximum xanthan gum production was 15.19 g/L (Fig. 2). The influence of temperature on xanthan gum production has been widely studied. Temperatures employed for xanthan gum production range from 25 to 34℃ [11,12].

3.1.3 Effect of pH on biomass and xanthan gum production

The effect of pH on xanthan gum production was studied with pH 5, 5.5, 6, 6.5, 7, 7.5, 8 of media was used. The study revealed that as pH increased from 5 to 6 the xanthan gum production also increased and decreased beyond pH 6. Thus the optimum pH for xanthan production was 6 and maximum yield was found to be 15.22 g/L. The optimum pH for biomass production is 6.5 and maximum production is 15.23 g/L (Fig. 3). Many studies have shown Most authors (Gumus et al. [12], Kerdsup et al., 2009; Psomas et al. [13]; Silva et al. [11]) that neutral pH is the optimum value for growth of *X. campestris* as the pH decreases from neutral pH to values close to 5 owing to acid groups present in xanthan (Borges et al. [14]).

3.1.4 Effect of different carbon sources on biomass and xanthan gum production

The production of biomass and xanthan gum was studied using various carbon sources like starch, sucrose, glucose, lactose, and maltose. The most suitable carbon source for biomass and xanthan gum production was sucrose and the maximum biomass production was found to be 24.01 g/L whereas the maximum xanthan gum production was obtained as 15.21 g/L (Fig. 4). The fermentation using *X. camprestris* and sucrose as the carbon source was best combination for maximum xanthan production [15,16].

3.1.5 Effect of different nitrogen sources on biomass and xanthan gum production

The biomass and xanthan production obtained by the *X. campestris* using various nitrogen sources like peptone, yeast extract, ammonium sulphate, ammonium nitrate, potassium nitrate were studied. Peptone showed the maximum biomass production as 23.91 g/L whereas the most suitable nitrogen source for xanthan gum production was yeast extract and the maximum production was found to be 15.17 g/L (Fig. 5). Peptone was the most suitable for maximum xanthan production [9,11].

3.2 FT-IR Spectra of Xanthan Gum

Functional group of synthesized xanthan gum was identified by FT-IR spectra. The FT-IR spectrum of synthesized xanthan gum is shown in Fig. 6. The broad absorbance peak at 3432.48 cm-1 indicates the hydrogen bonded –OH group.

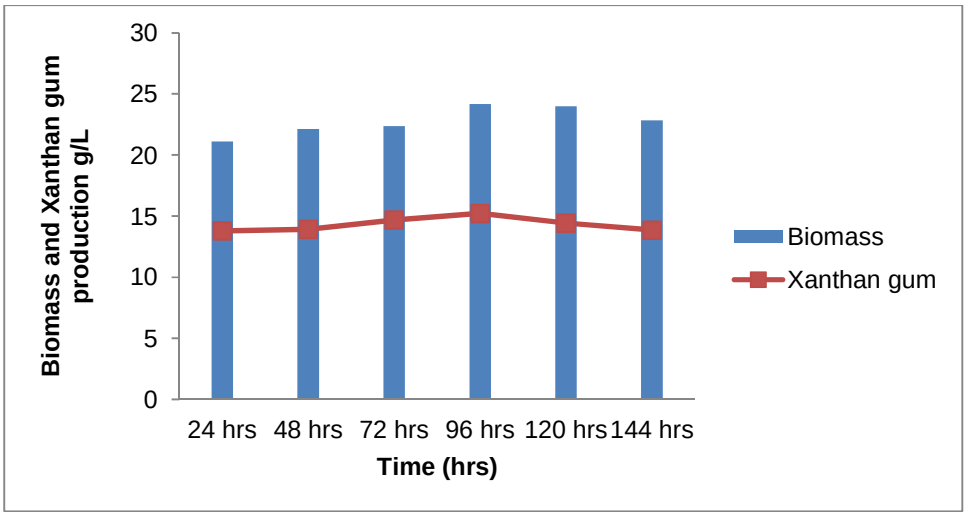

Fig. 1. Effect of different Incubation time on biomass and xanthan gum production

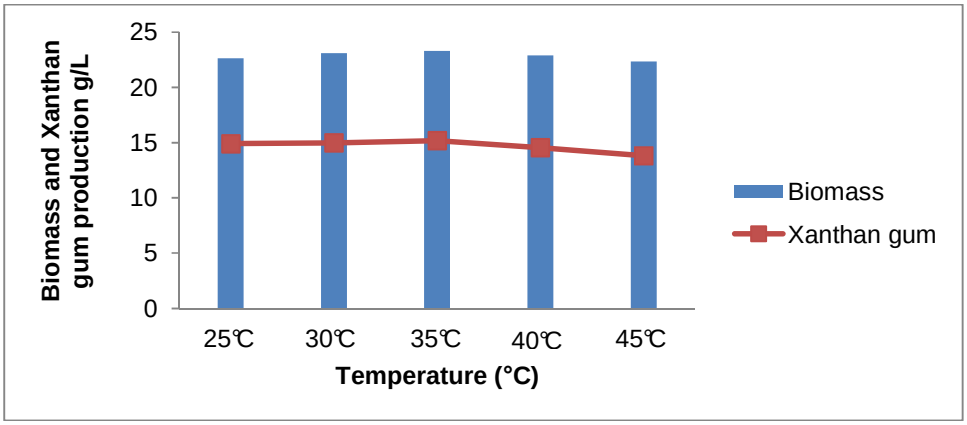

Fig. 2. Effect of temperature on biomass and xanthan gum production

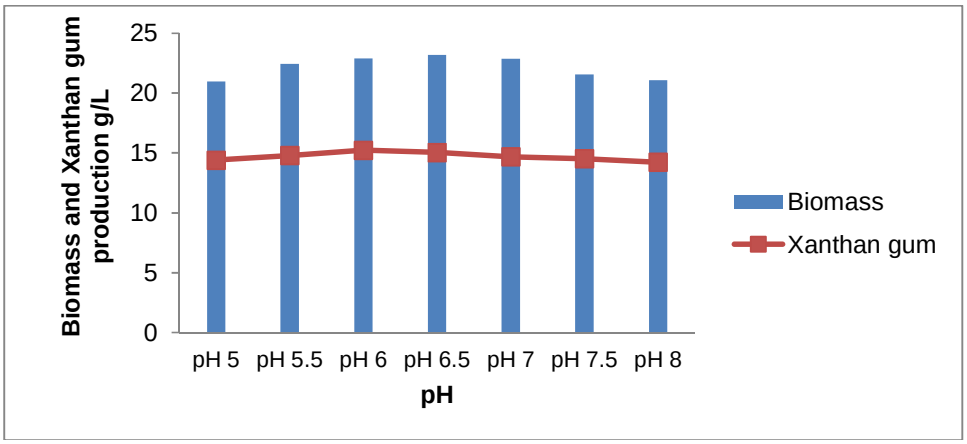

Fig. 3. Effect of pH on biomass and xanthan gum production

The absorbance peak at 2920.18 cm-1 indicates the presence of C-H bending from C-H2 and C-H3 vibration. The absorbance peak at 1729.26 cm-1 gives the information of presence of acetyl group and another peak at 1613.52 cm-1 indicate the presence of pyruvate group. The peaks

present at 1418.71 cm-1 and 1244.14 cm-1 are due to the carboxylate (-COO- group) asymmetric stretching and acetate (-C=O group) deformation respectively. Signals between 1100 cm-1 to 900 cm-1 were due to C-O stretching. The peak at 454.26 cm-1 is due glycoside bending groups (Fig. 6). The FT-IR spectrum of the Xanthan gum produced in this study followed the same pattern of emission bands shown in earlier study [4].

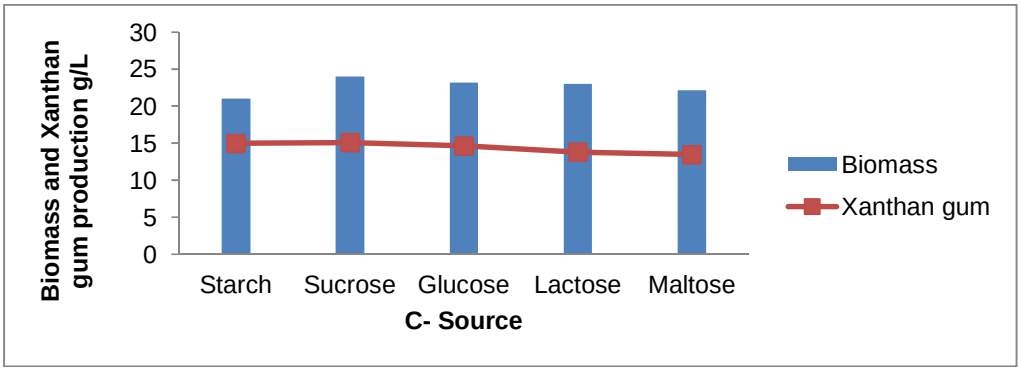

Fig. 4. Effect of different carbon sources on biomass and xanthan gum production

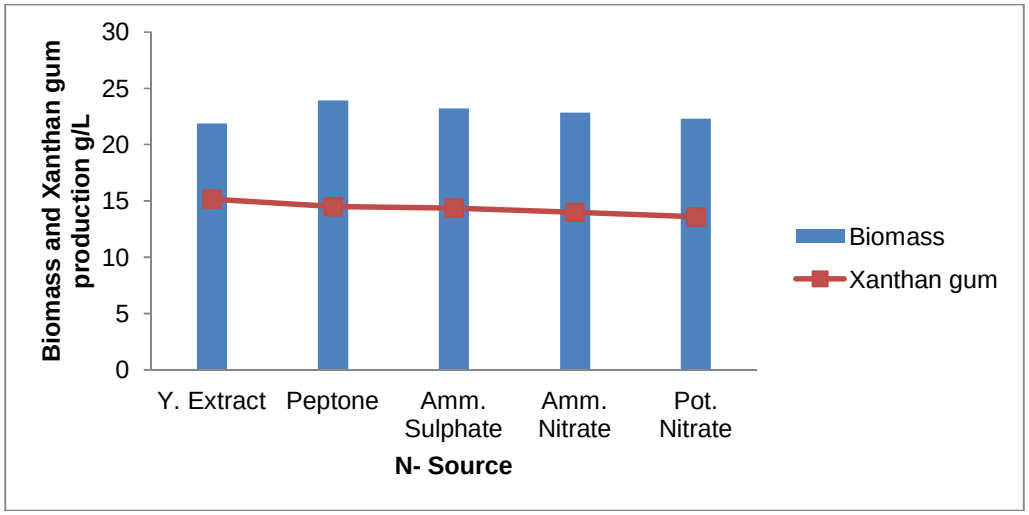

Fig. 5. Effect of different nitrogen sources on biomass and xanthan gum production

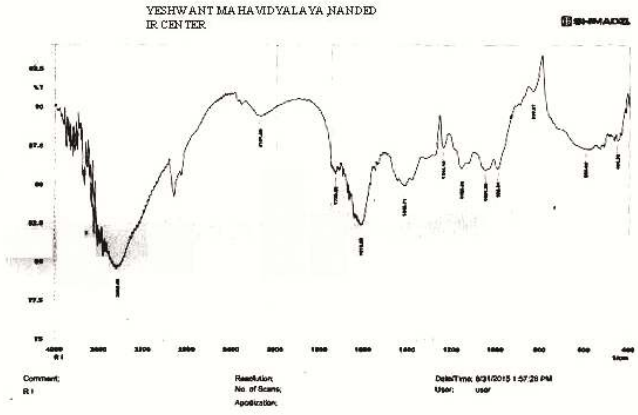

Fig. 6. FT- IR spectra of xanthan gum synthesized by using strain xan 22

The characterization of production of biomass and xanthan gum synthesized by *Xanthomonas* sps. was studied using various biochemical activities. This study will potentially important for bacterial application in different industrial areas and will also helpful to design an experiment for maximum production of xanthan gum.

4. CONCLUSION

This study focused on the relationship and characterization of biomass and xanthan gum production using various environmental and nutritional parameters. Incubation time of 96 hrs at 35℃ temperature with pH 6.5 were obtained to be optimal environmental conditions and sucrose as a carbon source while yeast extract as a nitrogen source were found to be optimal nutritional conditions for maximum production of xanthan gum.

ETHICAL APPROVAL

No ethical issues are associated with this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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