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Research Article

Optimization of Xanthan gum production by *Xanthomonas campestris*: Medium and culture conditions

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ABSTRACT:
Four strains of *Xanthomonas campestris* were isolated and screened for xanthan gum production and optimization of cultural and nutritional parameters. These four strains were isolated from Nanded district of Maharashtra. The characterization of the isolated strains was carried out by various morphological and biochemical tests. The study revealed that the maximum production of xanthan gum was recorded at 96 hrs for all four strains. The temperature of 35^0C and pH 6 was found to be optimum for the xanthan gum production. The medium optimization revealed that sucrose was most suitable carbon source and yeast extract was preferred nitrogen source for optimum xanthan gum production.

Keywords: Optimization, Xanthan gum, Xanthomonas campestris, nanded

INTRODUCTION:

The *Xanthomonas campestris* strain produced xanthan gum as a pathogenicity factor involved in host parasite interaction during pathogenesis(1). It is an anionic heteropolysaccharide discovered in late 1950s(2). Its concentration as an exopolysaccharide synthesized by the strains of *Xanthomonas* play an important role in pathogenicity of this species (3,4). Cultural parameters were considered as important factors that governs xanthan gum production. Xanthan gum production is influenced by the factors such as time, temperature, pH, carbon sources and nitrogen sources (5,6). Xanthan gum has wide application in food industries and is used as gelling agent, thickener and emulsion stabilizer. It is combined with galactomannans for the use as gelling agent due to its weak gel structure (7). Many studies shows production of xanthan gum produced from the strains obtained from culture collections. This study reports the isolation of wild strains from various sources and location of Nanded district in Maharashtra, screened for production and characterization of cultural parameters for xanthan gum.

MATERIALS AND METHODS:

Isolation of *Xanthomonas species*:

Four strains of *Xanthomonas campestris* isolated, from the trees of *Citrus lemona* from different location of Nanded district of Maharashtra were used in this study. Isolation and identification of these strains was done by following standard methods. The isolated strains were purified and maintained on YDC (Yeast extract – 1gm, D-glucose – 2gm, Calcium carbonate- 2gm) slants. These isolated cultures were further examined for morphological characteristics like colony characteristics, Gram staining, cell morphology,

cell motility (8). Biochemical test such as Aesculin test, Starch hydrolysis, Tween 80 lipolysis, H_2S production, Urease production, Milk proteolysis, Gelatin liquefaction, Oxidase test were also carried out.

Production media for xanthan gum:

The media used for the production of xanthan gum contained D- glucose – 20gm, Yeast Extract – 3gm, K_2HPO_4 – 2gm, $MgSO_4.7H_2O$– 0.1 gm, Distilled water – 1000ml. Fifty ml of the medium was taken in each 100 ml Erlenmeyer flask.

Optimization of cultural parameters for xanthan gum production:

1. Optimum incubation time for xanthan gum production:

After autoclaving, each 100 ml Erlenmeyer flask containing 50 ml of medium was inoculated with four different *Xanthomonas campestris* culture as *Xan*1, *Xan* 4, *Xan* 9 and *Xan*13.The optimum time for xanthan gum production was determined by using different time intervals as 1st day, 2nd day, 3rd day, 4th day, 5th day and 6th day by keeping all other process parameters constant.

2. Optimum temperature for xanthan gum production:

The autoclaved flasks containing 50 ml of production medium were inoculated with the culture of selected strains (*Xan*1, *Xan* 4, *Xan* 9 and *Xan* 13) and incubated at different temperature ranges from 25^0C, 30^0C, 35^0C,40^0C, 45^0C and 50^0C by keeping all other cultural conditions constant.

3. **Optimum pH for xanthan gum production:**

For determination of optimum pH for xanthan gum production different pH media of 5, 5.5, 6, 6.5, 7 and 7.5 were used. Each flask containing 50ml of xanthan gum production medium was adjusted to desired pH by using 1N HCL or 1N NaOH. The flasks were autoclaved at 15 lbs for 20 min and then inoculated with four different culture of *Xanthomonas campestris* as *Xan* 1, *Xan* 4, *Xan* 9 and *Xan* 13. The flasks were incubated by keeping all other process parameters constant.

4. Effect of carbon sources on xanthan gum production:

All flasks containing 50ml of xanthan gum production media with different carbon sources such as starch, sucrose, lactose and maltose instead of glucose with same concentration as 2% w/v was used to study the effect of carbon sources on xanthan gum production. The flasks were autoclaved at 15 lbs for 20 min. and then inoculated with culture of *Xan* 1, *Xan* 4, *Xan* 9 and *Xan* 13. These flasks were incubated by keeping all other conditions constant.

5. Effect of nitrogen sources on xanthan gum production:

The flasks containing 50ml of medium with different nitrogen source such as yeast extract, ammonium nitrate, ammonium sulphate, potassium nitrate and peptone at a concentration of 0.5% w/v. Four cultures of *Xanthomonas campestris* as *Xan* 1, *Xan* 4, *Xan* 9 and *Xan* 13 was inoculated in these different flasks and incubated at 35^0C for 48hrs by keeping all other conditions constant.

Extraction of xanthan gum:

All flasks were subjected to centrifuge for the extraction of xanthan gum. The medium was centrifuged at 10,000 rpm for 15 min and two fractions were formed in the centrifuge tube. The supernatant containing xanthan gum was collected and mixed with 2-3 volume of ethanol by continuous shaking to precipitate the xanthan gum. The obtained precipitate was recentrifuged at 6000rpm for 15 min and then residue as a xanthan gum was collected and transferred in preweighed microcentrifuge tube. To obtain dry weight of xanthan gum, this microcentrifuge tube containing xanthan gum was kept in hot air oven for drying at 60^0C for 20 hrs. Then this tube was cooled at room temperature and dry weight was measured. The determined dry weight gave the concentration of xanthan gum by calculating the dry weight of xanthan gum per litre of medium.

RESULT AND DISCUSSION:

Total four strains of *Xanthomonas campestris* were isolated from infected citrus leaves to study the production of xanthan gum. Xanthan gum production was affected by various cultural conditions and component of the medium like time, temperature, pH, carbon-sources (starch, sucrose, lactose and maltose) and nitrogen-sources (yeast extract, peptone, ammonium sulphate, ammonium nitrate and potassium nitrate).

Optimization of cultural parameters for xanthan gum production:

1. Effect of different incubation time on xanthan gum production:

To optimize the incubation time for xanthan gum production different time intervals were selected as 1st day, 2ndday, 3rd day, 4th day, 5th day and 6th day. From experiment it was observed that as time increased from 1st day to 4th day of incubation the production of xanthan gum was increased and there was decrease in production as time moved from 4th day to 6th day for all four strains. Hence optimum time for xanthan gum production was 96 hrs and maximum xanthan gum production by *Xan* 1 was 9.4 g/l, *Xan* 4 was 8.1 g/l, *Xan*9 was9.8 g/l and *Xan* 13 was 7.3 g/l as per

Table 1: Effect of different incubation time on xanthan gum production

Strains/Time hrs	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
Xan 1 (xg1 g/l)	4.7	5.9	7.9	9.4	8.2	6.8
Xan 4 (xg2 g/l)	3.5	6.2	6.9	8.1	6.7	4.4
Xan 9 (xg3 g/l)	4.9	6.8	8.4	9.8	7.7	6.2
Xan 13 (xg4 g/l)	4.1	6.3	6.7	7.3	5.3	4.2

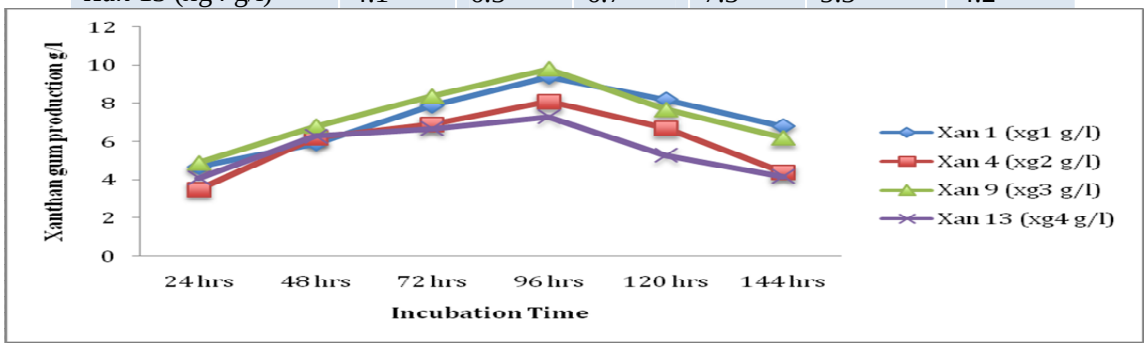

Fig 1: Effect of different incubation time on xanthan gum production

2. Effect of temperature on xanthan gum production:

The temperature ranges from 25^{0}C, 30^{0}C, 35^{0}C, 40^{0}C, 45^{0}C and 50^{0}C was used to determine the effect of temperature on xanthan gum production. From experiment it was found that xanthan gum was increased with increase in temperature from 25^{0}C to 35^{0}C and then reduction in production was found with increase in temperature from 35^{0}C to 50^{0}C. Hence it was cleared that the optimum temperature for xanthan gum production was 35^{0}C and maximum production of xanthan gum was 7.8 g/l synthesized by strain *Xan* 1, 8.5 g/l by strain *Xan* 4, 7.1 g/l by strain *Xan* 9 and 6.7 g/l by strain *Xan* 13 (**Fig.2**).

Table 2: Effect of different temperature on xanthan gum production

Strains/Temp^{0}C	25^{0}C	30^{0}C	35^{0}C	40^{0}C	45^{0}C	50^{0}C
Xan 1 (xg1 g/l)	3.7	5.7	7.8	6.4	6.2	4.8
Xan 4 (xg2 g/l)	3.5	6.2	8.5	8	6.7	5.4
Xan 9 (xg3 g/l)	3.9	5.8	7.1	5.8	4.7	3.9
Xan 13 (xg4 g/l)	4.7	5.3	6.7	6.3	5.1	4.3

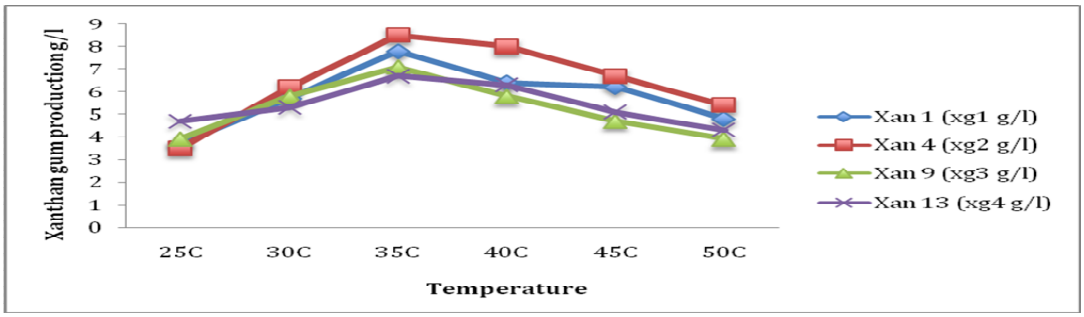

Fig 2: Effect of different temperature on xanthan gum production

4. Effect of pH on xanthan gum production:

Experiments were conducted to optimize the pH for xanthan gum production. Different pH ranges from pH 5, pH 5.5, pH 6, pH 6.5, pH 7 and pH 7.5 was used. The production of xanthan gum was increased in media from pH 5 to pH 6 and decreased in the media having pH above 6. Thus the optimum pH was 6 for the production of xanthan gum. The maximum production of xanthan gum was 6.4 g/l by strain *Xan* 1, 8.9g/l by strain *Xan* 4, 9.3 g/l by strain *Xan* 9 and 9.0 g/l by strain *Xan* 13 as per **Fig. 3**. Most of the authors reported that the neutral pH was the optimum value for the growth of *Xanthomonas campestris* as pH decreased from neutral value to close to pH 5 owed acid group present in the xanthan gum (6, 9).

Table 3: Effect of different pH on xanthan gum production

Strains/pH	pH5	pH5.5	pH6	pH6.5	pH7	pH7.5
Xan 1 (xg1 g/l)	3.3	5.4	6.4	6	5.2	4.1
Xan 4 (xg2 g/l)	4.5	7.2	8.9	7.8	5.7	4.4
Xan 9 (xg3 g/l)	4.9	7.8	9.3	8.8	6.7	3.7
*Xan*13(xg4g/l)	4.1	7.3	9	6.9	5.8	5.3

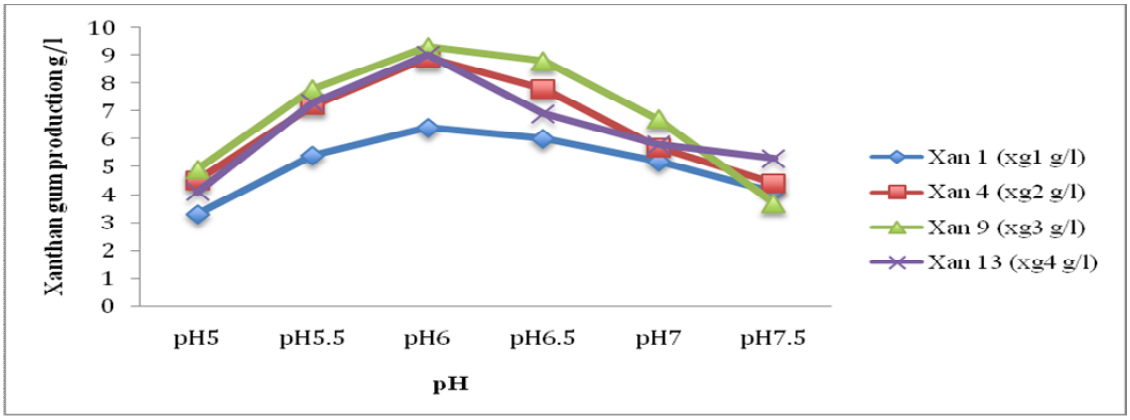

Fig. 3: Effect of different pH on xanthan gum production

5. Effect of different carbon sources on xanthan gum production:

The effect of different carbon sources on xanthan gum production was studied using various carbon-sources such as starch, sucrose, lactose, glucose and maltoseas previously described. The study revealed that sucrose was most suitable carbon source for maximum production of xanthan gum as compare to other carbon sources. Then maximum production of xanthan gum was 6.1 g/l, 8.8 g/l, 6.5 g/l, 10 g/l synthesized by *Xan* 1, *Xan* 4, *Xan* 9 and *Xan* 13 respectively as shown in **Fig 4**. Previous research also mentioned that fermentation using *Xanthomonas campestris* and sucrose as carbon source was best combination for maximum xanthan gum production (10,11).

Table 4: Effect of different carbon sources on xanthan gum production

Strains/CSource	Starch	Sucrose	Lactose	Glucose	Maltose
Xan 1 (xg1 g/l)	5.3	6.1	6.4	6.1	5.2
Xan 4 (xg2 g/l)	7.5	8.8	8.1	6.8	5.9
Xan 9 (xg3 g/l)	5.9	6.5	5.3	5.8	4.7
Xan 13 (xg4 g/l)	8.1	10	9.2	8.9	7.8

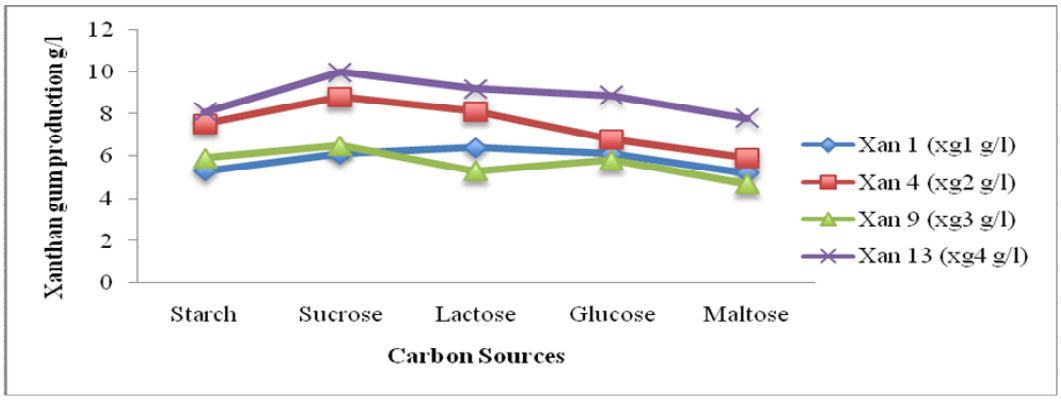

Fig. 4: Effect of different carbon sources on xanthan gum production

6. Effect of different nitrogen sources on xanthan gum production:

The effect of nitrogen sources on xanthan gum production was studied by conducting an experiment with various nitrogen sources like peptone, yeast extract, ammonium sulphate, ammonium nitrate and potassium nitrate. From this study, it was found that yeast extract showed maximum xanthan gum production as 7.8 g/l, 5.9 g/l, 9.9 g/l and 6.4 g/l synthesized by *Xan* 1, *Xan* 4, *Xan* 9 and *Xan* 13 respectively (**fig.5**). Thus it was cleared that yeast extract was most suitable nitrogen source for optimum production of xanthan gum.

Table 5: Effect of different nitrogen sources on xanthan gum production

Strains/N-sources	Peptone	YeastExtract	Amm.sulphate	Amm.nitrate	Pott.nitrate
Xan 1 (xg1 g/l)	6.3	7.8	5.4	4.6	5.8
Xan 4 (xg2 g/l)	4.5	5.9	4.7	5	3.7
Xan 9 (xg3 g/l)	8.9	9.9	9.3	8.7	6.9
Xan 13 (xg4 g/l)	5.1	6.4	5.9	6	5.8

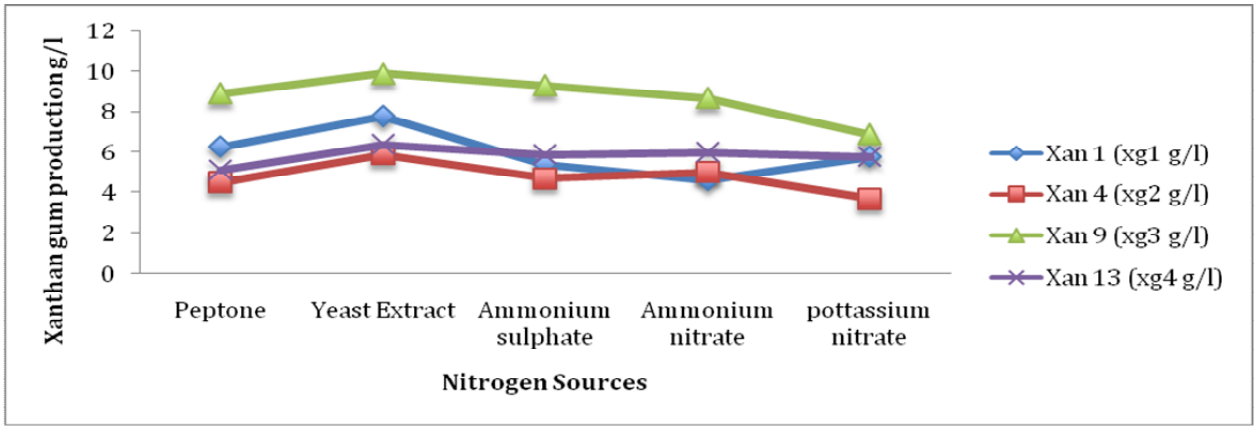

Fig. 5: Effect of different nitrogen sources on xanthan gum production

Production and characterization of xanthan gum was studied using various process parameters. This study will helpful to design an experiment for large scale production of xanthan gum and also useful for bacterial utilization in different industries as xanthan gum has wide industrial application.

For all four strains, the time, temperature and pH was 96 hrs, 35^0C and 6 was found to be optimal environmental conditions respectively and sucrose was most suitable carbon source while yeast extract was most suitable nitrogen source was recorded as optimal nutritional condition for maximum production of xanthan gum.

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