Fermentation Process and Nutrition Study of Xanthomonas campestris and Xanthomonas malvacerum in xanthan gum production

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The study deals with comparative analysis of xanthan gum production, pyruvate, acetate and protein content of *Xanthomonas campestris* MTCC 2286 and *Xanthomonas malvacerum*. The *Xanthomonas malvacerum* isolated strain could be useful in oil recovery process. *Xanthomonas malvacerum* has produced high level of xanthan gum (16.8g/l). It is observed from the results that addition of citric acid in the fermentation medium, increases xanthan gum concentration.

Keywords: Xanthomonas campestris, Xanthomonas malvacerum and xanthan gum

The isolation and screening of *Xanthomonas* strains from natural habitats is an important tool to improve xanthan gum production. Industrially produced microbial polysaccharides xanthan gum is the most important. It is produced by *Xanthomonas campestris* (Galindo, 1994a) and has applications in many industrial fields such as textiles, ceramics, agricultural production, food production, oil recovery (Kennedy & Bradshaw 1984). *Xanthomonas campestris* is phytopathogenic bacteria which cause black rot in crucifers (Sutton & Williams 1970).

Xanthan gum production has been performed with strains from culture collections, especially *Xanthomonas campestris* NRRL B-1459. There has been relatively little work on production by wild strains (Torrestiana *et al.*, 1990). Ramirex *et al.*, 1988) suggested virulence in plants was a good indicator of gum production. There are very few reports dealing with the characterization reported the isolation of 21 strains of *Xanthomonas campestris* from tropical regions and the viscosity of the fermentation broth was measured in a Brookfield viscometer. Comparison of wild type *Xanthomonas* strains with *Xanthomonas malvacerum* isolates has not been previously reported. The aim of the present work is selection, characterization and production of xanthan gum from *Xanthomonas malvacerum* strain isolated form infected cotton leaves with *Xanthomonas campestris* MTCC 2286.

MATERIALS AND METHODS

Isolation of strain

Xanthomonas malvacerum was isolated from infected cotton leaves. This isolate was assessed by a shake flask for xanthan productivity test (Galindo *et al.*, 1993). A variant of the collected strain *Xanthomonas campestris* MTCC 2286 was used for comparative studies.

Fermentation Media

YM medium was used for inoculum development of *Xanthomonas campestris* (Flores *et al.*, 1994).

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The composition of the production medium was (g/l): glucose 24; peptone 0.8; KH_2PO_4 3.068; $(NH_4)_2PO_4$ 0.6973; citric acid 1.3; Mg SO_4 0.23; FeCl₃ 0.0014; CaCO₃ 0.0024; H₃Bo₂ 0.0048; ZnO₃ 0.0072; pH was adjusted to 7.2.

Isolation of Xanthomonas malvacerum from infected leaves

The blight infected leaves were collected from the cotton field. The diseased spots were dissected out, the surface was sterilized with mercuric chloride (0.1% W/V), than thoroughly washed with sterile distilled water and transferred aseptically to Hoitink and Sindern's (HS) medium plates. The plates were incubated at 29° C for three days. A yellow bacterium was grown from the leaf spots and it was confirmed by the routine bacteriological test (Bergey's manual of systematic bacteriology 1984).

Fermentation process

Wild and isolated strain were cultured in duplicate in 500ml production medium in 2000ml flasks for 48hrs at 29° C in an incubator shaker at 200rpm. The inocula 10% V/V was used for xanthan production.

Analytical procedures

Bacterial cell mass was determined by dry weight method at various time intervals. Xanthan gum was recovered by precipitation with two volumes of isopropyl alcohol using 2% W/W potassium chloride as electrolyte. The product was dried in an oven at 40° C for 24hrs.

The content of polysaccharide per gram of product was determined according to the sulphuric acid – resorcinol method (Graham 1971). Estimation of glucose concentration was determined by DNS method. Pyruvic acid was estimated by the enzymatic method of Hadjivassilioic and Rieder (1968). Acetyl residues were determined by the colorimetric method reported by Mc Comb and Mc Cready (1957). Nitrogen was determined by the Lowry method.

RESULTS AND DISCUSSION

The time course of xanthan biosynthesis, cell concentration and glucose consumption was shown in Table 1. The yield of xanthan productivity from 0.04 to 0.05 (g xanthan per g of glucose

Time (hrs)	Citric acid added(g/l)	Cell Conc. (g/l)		Xanthan Conc. (g/l)		Glucose Consumption (%)		Gm of xanthan/gm of cell		Gm of xanthan /gm of glucose consumption	
		А	В	А	В	А	В	А	В	А	В
24	0.00	2.0	1.5	6.0	7.0	12.0	20.0	3.0	4.6	0.5	0.35
32	0.50	2.4	2.2	8.8	14.0	16.0	28.0	3.6	6.3	0.54	0.50
40	1.00	2.6	2.4	13.6	15.5	18.0	30.0	5.2	6.4	0.75	0.51
48	1.50	2.6	2.4	15.5	16.0	19.0	31.0	5.9	6.6	0.81	0.51
56	2.0	2.8	2.5	17.5	17.5	20.0	32.0	6.2	7.0	0.87	0.54
64	2.5	2.8	2.5	18.5	18.5	20.0	32.0	6.6	7.4	0.92	0.57
72	3.0	2.9	2.5	19.0	18.5	21.0	33.0	6.3	7.4	0.88	0.56

Table 1. Effect of citric acid addition on xanthan production

A - Xanthomonas campestris; B - Xanthomonas malvacerum

consumed) in Xanthomonas malvacerum. But the production of xanthan in Xanthomonas campestris was 0.21 to 0.37 g of xanthan /gm of glucose. A corresponding increase in specific xanthan production from 1.25 to 1.42g of xanthan /g of cell and from 1.1 to 1.54 gm of xanthan /gm in Xanthomonas malvacerum and Xanthomonas campestris respectively. Jana and Ghosh (1997) reported that due to better oxygen transfer and mixing behaviour in the fermentation broth the yield of xanthan (g xanthan per gm of glucose consumed) also increased from 0.49 to 0.59.

The effect of citric acid on xanthan production was observed in Table 2. Different concentration of citricacid was added after 24hrs of fermentation in 8hrs interval (ranges from

Time (hrs)	Cell Conc. (g/l)		Xanthan Conc. (g/l)		Glucose Consumption (%)		Gm of xanthan/gm of cell		Gm of xanthan/gm of glucose consumption	
	А	В	А	В	А	В	А	В	А	В
12	1.5	2.0	2.0	2.5	40.0	8.0	1.3	1.25	0.05	0.31
24	2.0	4.2	2.5	6.5	54.0	20.0	1.24	1.54	0.04	0.32
36	2.2	8.5	3.0	12.0	62.0	32.0	1.36	1.41	0.04	0.7
48	2.5	10.0	3.5	15.0	68.0	40.0	1.4	1.5	0.05	0.37
60	2.8	12.5	3.8	15.5	74.0	52.0	1.35	1.25	0.05	0.29
72	2.8	14.0	4.0	16.0	76.0	61.6	1.42	1.14	0.05	0.25
84	2.9	14.4	4.0	16.0	78.0	72.0	1.37	1.11	0.05	0.22
96	3.1	14.6	4.0	16.8	80.0	77.6	1.29	1.15	0.05	0.21

 Table 2. The data shown values for cell density product, glucose concentration of shake flask run at pH 7.2 in Xanthomonas campestris and Xanthomonas malvacerum

A - Xanthomonas campestris; B - Xanthomonas malvacerum

0.50g/l to 3.0g/l). Citricacid concentration above 3g/l did not improve xanthan production. Citricacid was totally consumed in 24hrs in all case except when the initial concentration of citricacid was 6 or 8g/l. Citric acid above 8g/l however, prevented both cell growth and xanthan synthesis. Increase in xanthan concentration was observed to be dependent on the amount of citricacid added

(Table 2). Our results were correlated with the finding of Jana and Ghosh (1997) increased in xanthan concentration from 10.4g/l to 18.6g/l. The chemical composition of the polymers obtained from the *Xanthomonas campestris* with *Xanthomonas malvacerum* isolates are compared in Table 3. The xanthan showed a range in acetyl and pyruvic contents, with values similar to those

 Table 3. Chemical composition of the xanthan gum polymers obtained from

 Xanthomonas campestris and Xanthomonas malvacerum

Strain	Pyruvate (%)	Acetate (%)	Protein (%)
Xanthomonas malvacerum	2.50	7.5	1.2
Xanthomonas campestris	5.85	3.2	1.5

reported from other isolates (Sanchez et al., 1997). Low pyruvate content was determined in the product from Xanthomonas malvacerum. These could be especially in xanthan applications related to oil recovery processes. Kleinitz et al., (1989) reported that in oil recovery process xanthan with low pyruvate content showed lower adsorption over the soil. Total nitrogen value was determined in the products from Xanthomonas malvacerum and Xanthomonas campestris (1.2% and 1.5%). Sanchez et al., (1997) reported that the range of nitrogen content in the xanthan from four isolates (1.01% to 3.04%). High proteinaceous nitrogen contents could restrict the use of xanthan in application such as food additives or cosmetics.

Characterization showed that the two selected isolates of *Xanthomonas campestris* and *Xanthomonas malvacerum* have high potential for xanthan production. Isolate *Xanthomonas malvacerum* could be useful for the oil recovery process because they exhibited low pyruvate content. In the screening of *Xanthomonas malvacerum* isolate, the productivity test was compared with *Xanthomonas campestris* MTCC 2286, and to decide *Xanthomonas malvacerum* has potential for xanthan production.

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