African Journal of Biotechnology Vol. 10(74), pp. 16924-16928, 23 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.973
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Production and characterization of the polysaccharide ‘’xanthan gum’’ by a local isolate of the bacterium *Xanthomonas campestris*

M. B. I. Kassim

Department of Biology, College of Education, Mosul University, Mosul, Iraq. E-mail: dr_mbikassim@yahoo.com. Tel: +9647704122589.

Accepted 20 January, 2011

A local isolate of the bacterium *Xanthomonas campestris* was obtained from infected cabbage leaves. Microbiological and biochemical tests were made to confirm its identification. The isolate was coded as *X. campestris* MU1. Growth and xanthan production reached their highest levels (1.65 and 5.41 g/l) after 7 and 8 days of incubation, respectively, in a chemically defined medium. Sucrose was shown to be the best carbon source for xanthan production. Thin layer chromatography of the produced xanthan showed that, it composed glucose, mannose and glucouronic acid, in addition to pyruvic and acetic acids. The results indicate that, *X. campestris* MU1 is very much related to *X. campestris* NRRL-B 1459.

Key words: *Xanthomonas campestris*, xanthan gum, microbial polysaccharides.

INTRODUCTION

Xanthan or xanthan gum, presently the most important microbial polysaccharide (Paul et al., 1986) is produced by microbial fermentation with the bacterium *Xanthomonas campestris* (Galindo, 1994). Most *Xanthomonas* strains are relatively common phytopathogenic bacteria which mainly infect crucifers and cause black rot which has economic importance in crop damage in certain areas (Torrestiana et al., 1990; Ramirez et al., 1988; Vicente et al., 2001; Sahin et al., 2003). *X. campestris* also infects tomato (Shenge et al., 2007).

Xanthan structure is based on cellulose back bone having alternate glucosyl residues substituted by a triasaccharide chain of D-mannose, D-glucouronic acid and a terminal D-mannose. It is variably substituted by o-acetyle and pyruvic acid ketal groups (Kennedy and Bradshow, 1984). Xanthan gum has many applications in food, cosmetics, pharmaceutical and oil industries because of its high viscosity (Bradford and Baid, 1983; Sandvic and Maeker, 1977; Cottrel and Kang, 1978). Most of the work regarding to production of xanthan gum has been reported with strains from cultures collection mainly *X. campestris* NRRL-B 1459 or ATCC 13951 and their derivatives. Reports on the isolation of wild strains of *X. campestris* having potentiality to produce xanthan gum are very limited. Scamparini and Rosato (1987), Torrestiana et al. (1990) and Ramirez et al. (1988) have reported the isolation of several wild *Xanthomonas* strains some of which were promising as potential producers of xanthan gum. In this article, production of xanthan by a local isolate of *X. campestris* and characterization of the produced xanthan are reported.

MATERIALS AND METHODS

Microorganism

A local isolate of *X. campestris* was obtained from infected cabbage leaves *(Brassica oleracea);* leaves showing the yellow necrotic lesion characteristic of *X. campestris* were collected randomly from a cabbage field near Mosul city. The diseased leaf tissues were cut into small pieces, soaked in 5 ml sterile distilled water and kept at the laboratory for 24 h. The resulting suspension was streaked onto malt-yeast (MY) extract agar. The plates were incubated at 28 ± 1°C for 4 days. Single bacterial colonies displaying characteristics of *Xanthomonas* spp were selected and purified by re-streaking on MY agar. A yellow convex mucoid colony was submitted to standard microbiological and biochemical tests (Bergeys, 1974). The cultures were maintained on MY agar slants at 4°C and were subcultured every two weeks.

Inoculation and culture media

The iocula were prepared by transferring cells from 72 h MY agar slants incubated at 28 ± 1°C to 250 ml Erlenmeyer flasks containing 50 ml of MY broth which consists of (g/l) glucose, 10; peptone, 5;

yeast extract, 3; malt extract, 3. The pH was adjusted to 7.0 before autoclaving at 121°C for 20 min. (De Vuyst et al., 1987). Incubation was in shaker incubator at 150 rpm and 28 ± 1°C for 48 h. These cultures were used for seed production medium at 2%. The production basic medium composed of the following (g/l), K2HPo4, 5; MgSo4.7H2O, 0.1; yeast extract, 0.5 and urea, 0.4; glucose, 20 was added as a carbon source unless otherwise stated and sterilized separately (Nitsche and Thomas, 1995). The pH was adjusted to 7.3 before sterilization. The medium was distributed into 250 ml Erlenmeyer flasks each with 50 ml and sterilized, after cooling; the flasks were seeded with the prepared inoculum of *X. campestris* and incubated in shaker incubator at 150 rpm and 28 ±1°C.

Analytical methods

During fermentation cycles, at regular time intervals, duplicated samples were withdrawn and analyzed for pH, growth, levels of xanthan, residual sugar and characterization of the produced xanthan. Growth was measured by cell dry mass determination. The samples were therefore, centrifuged and when necessary diluted with distilled water to decrease the viscosity. The centrifuged cells were washed twice with distilled water and centrifuged again. The precipitated cells were dried at 80°C for 24 h. The crude xanthan was precipitated from the supernatant by addition of two volumes of cold acetone, then the mixture was centrifuged and precipitate was collected and dried at 50°C until constant weight was achieved. Residual sugar was determined by the phenol sulphuric acid method (Dubois et al., 1956) using glucose as standard. Characterization of xanthan produced by the wild strain was made according to Kawahara and Obata (1998) with little modification. The purified xanthan was obtained by repeatedly dissolving xanthan in distilled water and reprecipitation with two volumes of cold acetone. 100 mg of pure xanthan was hydrolysed with 10 ml of 2M trifluroacetic acid at 120°C for 2.5 h. The acid was removed by continuous evaporation at 50°C under reduced pressure. Pyruvic and acetic were extracted from the hydrolysate by ether. The water solution obtained was neutralized with $BaCo_3$. After removal of $BaCo_3$, the volume of the filtrate was concentrated to 1 ml by evaporation at 50°C under reduced pressure. The volume of ether portion was also concentrated to 1 ml by evaporation at 50°C.

The sugar and organic acids were analyzed by thin layer chromatography on Kieselgel 60 TLC 20 × 20 cm and 0.25 mm thickness Merk. The sugars were analyzed with solvent system N-butanol: ethanol: water (5: 5: 4 by vol.). The thin layer chromatography (TLC) plates were dried at 60°C and the sugars were detected by spraying chromatograms with 5% ethanolic sulphuric and heating in the oven at 100°C until clear sugars spots appeared (Amemura et al., 1985). The organic acids were analyzed with solvent system ethanol: ammonium: water (70: 4: 16 by vol.) and chromatograms sprayed with glucose-aniline solution and heated at 125°C for 10 min until clear spots of the acids appeared (Touchston and Dobbins, 1978).

For measurement of viscosity, the purified xanthan solutions were diluted with distilled water. For each concentration, the viscosity of all samples were measured with Ostwald-Fenske viscometer.

RESULTS AND DISCUSSION

Isolation, identification and characterization of *X. campestris*

A yellow mucoid bacterium was consistently isolated from cabbage leaves samples with typical bacterial leaf spot symptoms. The bacterium was gram-negative, rod shaped, motile, aerobic, catalase positive and oxidase negative. In addition, the bacterium was also subjected to a number of other biochemical tests (Bergeys, 1976). Therefore, the bacterium was confirmed as a local strain of *X. campestris* and it was coded as *X. campestris* MU1.

Production of xanthan gum

The results of xanthan production and growth changes in sugar uptake and final pH of *X. campestris* MU1 cultures within an incubation period of 8 days in production medium are presented in Table 1. *X. campestris* MU1 produced 2.66 g/l xanthan after 2 days of incubation. Xanthan production continued to increase up to the end of incubation (8 days) where maximum xanthan production was obtained (5.41 g/l). The same trend was observed in biomass production where maximum biomass (1.65 g/l) after 7 days of incubation. Sugar consumption was a reflection of growth and xanthan production. The incubation period of 8 days in order to give maximum production of xanthan is considered to be very long in comparison to other *X. campestris* strains. Souw and Demain (1979) stated that, maximum xanthan production was attained after only 5 days of incubation of *X. campestris* NRRL-B 1459. Torrestiana et al. (1990) showed that, 0.8 g/l xanthan was obtained after 48 h of incubation by different strains of *X. campestris*. These differences in xanthan production by different strains of *X. campestris* could be attributed to strain variation. The decrease in pH after fermentation is normally due to the accumulation of xanthan gum in culture broth. Xanthan is acidic in nature, as it contains organic acids in its structure.

Effect of carbon source

Xanthan gum production by different strains of *X. campestris* using different carbon sources has been investigated by many researchers. As shown in Table 2, when *X. campestris* MU1 was cultured in various sugars as a carbon source at the concentrations of 2% in production medium, it could produce xanthan at different levels. Highest xanthan, 6.8 g/l, was produced when sucrose was used as a carbon source. This result was in agreement with Souw and Demain (1979) and Kawahara and Obata (1998) who stated that, maximum xanthan production was obtained when sucrose was used as a carbon source using *X. campestris* NRRL-B 1459 and *X. campestris* pv. translucens, respectively. Also, glucose, fructose and mannose were good sources of carbon for the production of xanthan by *X. campestris* MU1. On the other hand, lactose and xylose did not support high production of xanthan by *X. campestris* MU1. Kawahara

Table 1. Effect of incubation period on Xanthen production, growth, changes in pH of the medium and residual glucose for *X. campestris* MU1.

Incubation (days)	Biomass (g/l)	Xanthan (g/l)	Residual sugar (g/l)	Final pH
2	0.33	2.66	14.3	7.01
3	0.41	3.48	13.1	6.8
4	0.71	4.10	11.4	6.2
5	0.96	4.32	9.2	6.1
6	1.03	5.06	7.36	5.9
7	1.65	5.37	5.7	5.7
8	1.42	5.41	5.1	5.61

The data are the average of duplicate samples.

Table 2. Effect of carbon source on production of Xanthan, biomass and final pH for *X. campestris* MU1

Carbon source	Biomass(g/l)	Xanthan (g/l)	Final pH
Sucrose	2.2	6.8	5.8
Glucose	1.5	6.1	5.1
Fructose	1.7	5.7	5.9
Galactose	1.9	5.2	6.5
Mannose	2.5	5.5	6.2
Rhamnose	0.9	2.8	6.6
Cellobiose	2.6	4.1	5.8
Lactose	1.4	1.9	6.4
Xylose	0.7	1.1	6.9

The data are the average of duplicate samples

and Obata (1998) found that, no xanthan was produced when xylose was used as a carbon source. Production of xanthan by *X. campestris* MU1 was very poor when lactose was used as a carbon source. This may be due to the low activity of galactosidase produced by *X. campestris* MU1 to break lactose into glucose and galactose (Walsh et al., 1984; Fu and Tseg, 1990).

Characterization of xanthan

The viscosity of xanthan gum solution produced by *X. campestris* MU1 was 4.1 cp at 0.1% concentration. This viscosity was lower than that of the commercial xanthan (Kesterol) which was 4.93 (Torres et al., 1993). Thin layer chromatography of acid hydrolysate of purified xanthan produced by *X. campestris* MU1, (Figure 1a) revealed the presence of glucose, mannose and glucouronic acid. The spots of these sugars coincided with spots of standard glucose, mannose and glucouronic acid. Also, thin layer chromatography analysis of acetic and pyruvic acids showed the presence of these two acids (Figure 1b). The results of chemical composition of xanthan produced by *X. campestris* MU1 revealed by thin layer chromatography are in agreement with that of xanthan gum produced by *X. campestris* NRRL-B 1459 (Jansson et al., 1975; Melton et al., 1976). The overall results indicate that, *X. campestris* MU1 is very much related to *X. campestris* NRRL-B 1459.

The objective of this work was to have our own strains of the bacterium *X. campestris*. Attempts to improve the yield of xanthan by *X. campestris* MU1 either by changing the cultural conditions or by chemical or physical mutagenesis are in progress in our laboratory.

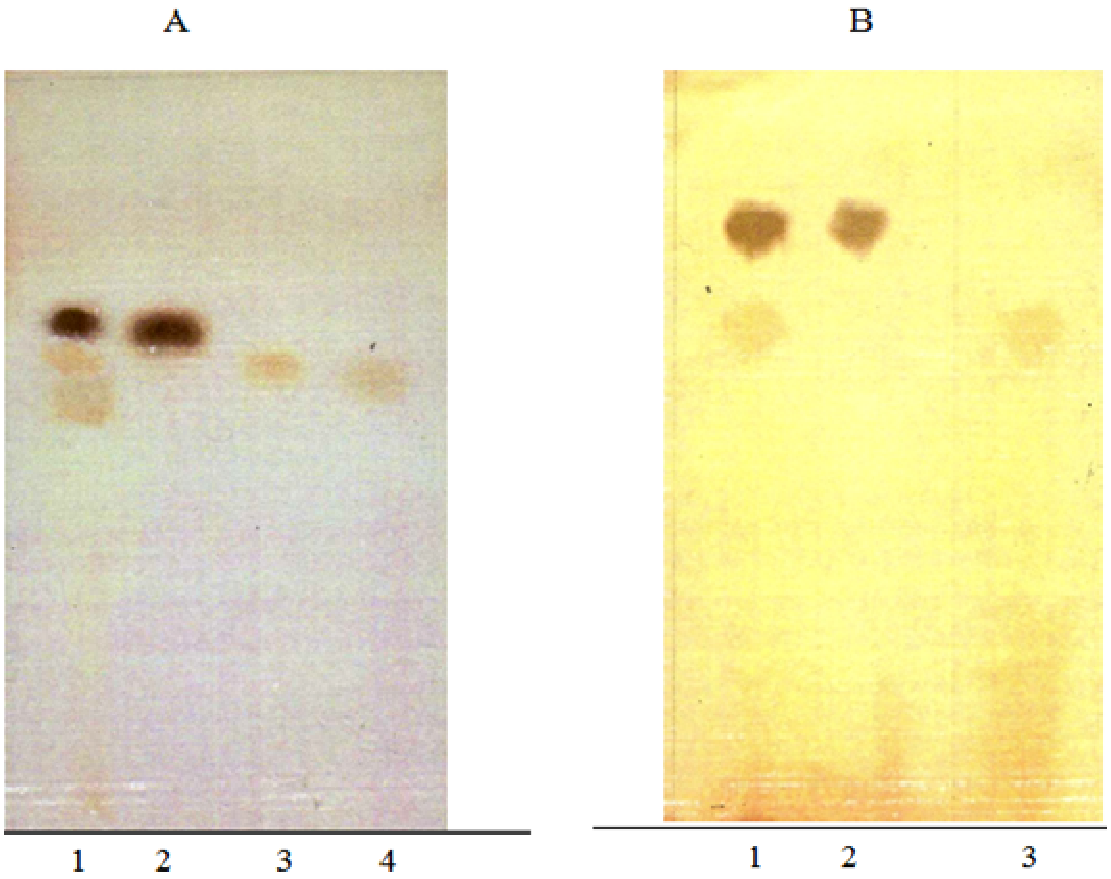

Figure 1. Thin-layer chromatography of the components of xanthan gum produced by *X. campestris* MU1. (A) 1, Xanthan sample; 2, glucose; 3, mannose; 4, glucuronic acid. (B) 1, xanthan sample; 2, pyruvic acid; 3, acetic acid.

Abbreviations:

MY, Malt-yeast; **TLC**, thin layer chromatography.

REFERENCES

Amemura A, Hashimoto T, Koizumi K, Utamura T (1985). Occurrence of extracellular (1, 2) – β – D glucose and (1, 2) – β – D gluco – oligosaccharides in Acetobacter. J. Gen. Microbiol. 131: 301-307.

Bergeys DH (1974). Bergey's manual of determinative bacteriology, 8th ed., Buchanan RE and Gibbons NE (ed). The Williams and Wilkins Co. Batimore USA.

Bradford PA, Baid J (1983). Industrial utilization of polysaccharide. In: Aspinall GO (ed) The polysaccharides, Academic Press, New York, 2: 411-490.

Cottrel IW, Kang KS (1978). Xanthan gum a unique bacterial polysaccharide for food applications. Dev. Ind. Microbiol. 19: 117-131.

De vuyst L, Van Loo J, vandamme E (1987). o – Step fermentation process for improved xanthan production by Xanthomonas campestris NRRL-B 1459 . J. Chem. Teel. Biotechnol. 39: 263-273.

Dubois M, Gilles KA, Hamilton JK, Roberts PA, Smith F (1956). Colorimetric method for the determination of sugars. Anal. Chem. 28: 350-356.

Fu JF, Tseng YH (1990). Construction of lactose utiliting Xanthomonas campestris and production of xanthan gum from whey. Appl. Environ. Microbiol. 56: 919-923.

Galindo E (1994). Aspects of the process for xanthan production. Trans. I. Chem. E. 72: 227-237.

Jansson PE, Kenne L, Lindberg B (1975). Structure of extracellular polysaccharide from Xanthomonas campestris. Carbohydr. Res. 45: 275-282.

Kawahara H, Obata H (1998). Production of xanthan gum and ice – nucleating material from why by X. campestris pv. Translucens. Appl. Microbiol. Biotechnol. 49: 353-358.

Kennedy JF, Bradshaw IJ (1984). Production properties and applications of xanthan. Prog. Ind. Microbiol. 19: 319-371.

Melton LD, Mindt L, Rees DA, Sanderson GR (1976). Covalent structure of the extracellular polysaccharide from Xanthomonas campestris: evidence from partial hydrolysis studies. Carbohydr. Res. 46: 245-257.

Nitsche M, Thomas WS (1995). Xanthan gum production by wild – type isolates of Xanthomonas campestris. World J. Microbiol. Biotechnol. 11: 502-504.

Paul F, Morin A, Monsan P (1986). Microbial polysaccharides with actual potential industrial application. Biotechnol. Adv. 4: 245-259.

Ramirez ME, Fucikovsky L, Garcia – Jimenez F, Quintero R, Galindo E (1988). Xanthan gum production by altered pathgenicity variants of *Xanthomonas campestris*. Appl. Microbiol. Biotechnol. 29: 5-10.

Sahin PA, Abbasi ML, Lewis ML, Zhang J, Miller SA (2003). Diversity among strains of Xanthomonas campestris pv. vitians from lettuce. Phytopathology, 93: 64-70.

Sandvic EL, Maeker JM (1977). Application of xanthan gum for enhanced oil recovery. Extracellular microbial polysaccharides . Am. Chem. Soc. Symp. Ser. 45: 242-264.

Scamparini ARP, Rosato YB (1987). Production of xanthan gum by

different isolates of Xanthomonas campestris. Proceedings: 4th European Congress on Biotechnology ed. Niejssel DM, Van der Meer RR and Luyben K, Ch. A.M. Amsterdam: Elsevier Sci. 1: 301-304.
Shenge KC, Mabagala RB, Mortensen CN (2007). Identification and characterization of strains of Xanthomonas campestris pv. vesicatoria from Tanzania by biology systems and sensitivity to antibiotics . Afr. J. Biotechnol. 6: 015 -022.
Souw P, Demain AL (1979). Nutritional studies on xanthan production by Xanthomonas campestris NRRL-B 1459. Appl. Environ. Microbiol. 37: 1186-1192.
Torres LG, Brito E, Galindo E, Choplin L (1993) . Viscous behaviour of xanthan aqueous solution from a variant strain of Xanthomonas campestris. J. Ferm. Bioeng. 75 : 58-64 .
Torrestiana B, Fucikovsky L, Galindo E (1990). Xanthan production by some Xanthomonas isolates. Lett. Appl. Microbiol. 10: 81-83.
Touchston JD, Dobbins MF (1978). Practic of Thin layer chromatography. A Wiley – Interscience Publication. John Wiley and Sons. new york, USA. pp. 182-186.
Vicente JG, Conway J, Robert J, Taylor JD (2001). Identification and origin of Xanthomonas campestris pv. campestris races and related pathovars. Phytopathology, 91: 492-499.