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Mutagenesis of *Xanthomonas campestris* and Selection of Strains with Enhanced Xanthan Production

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ABSTRACT

Xanthan gum is a microbial polysaccharide of great commercial importance as it has unusual rheological properties in solution and consequent range of applications. In this study, a series of mutants were isolated from *Xanthomonas campestris* PTCC 1473 by ethyl methanesulfonate mutagenesis. The polysaccharide yield of one mutant, XC1473E$_2$, was 30% better than that of the parent strain. It also showed higher xanthan formation and glucose consumption rates compared to the parent strain. Xanthan produced by the mutant had enhanced viscosity, higher pseudoplasticity and larger molecular weight. Since mutant XC1473E$_2$ appeared white on agar plates, it underwent pigment extraction with methanol. Contrary to the parent strain, the mutant showed no absorption at 443nm, i.e. the wavelength related to yellow pigment. This finding suggested that yellow pigmentation and normal xanthan biosynthesis are not necessarily concurrent. In general, mutant XC1473E$_2$ seems to be a strain with interesting characteristics for use in commercial production of xanthan. *Iran. Biomed. J. 7 (3): 91-98, 2003*

Keywords: Xanthan, *Xanthomonas campestris*, Mutation, Xanthomonadin

INTRODUCTION

Xanthan is an extracellular hetero-polysaccharide produced by the yellow-pigmented bacterium *Xanthomonas campestris* pv. campestris, a phytopathogen causing black rot in crucifers [1, 2]. The structure of xanthan consists of ß-1,4-linked D-glucose backbone carrying trisaccharide side chains composed of two mannoses and one glucoronic acid attached to alternate glucose residues in the backbone [3] (Fig. 1). The side chain is additionally modified by acetylation of the inner and sometimes outer mannosyl residues, and pyruvylation of the outer mannosyl residue [3-5]. However, the extent of acetylation and particularly pyruvylation can vary considerably depends on fermentation conditions, as well as strain variants [6-8]. Molecular weight of xanthan ranges from 0.9×10^6 to 1.6×10^6 Daltons (Da) that is dependent on microbial source and fermentation conditions [9, 10].

Xanthan is the most commercially accepted microbial polysaccharide that is the subject of numerous studies. Worldwide consumption of xanthan is approximately 23 million kg/year estimated to grow continuously at an annual rate of 5%-10% [11, 12].

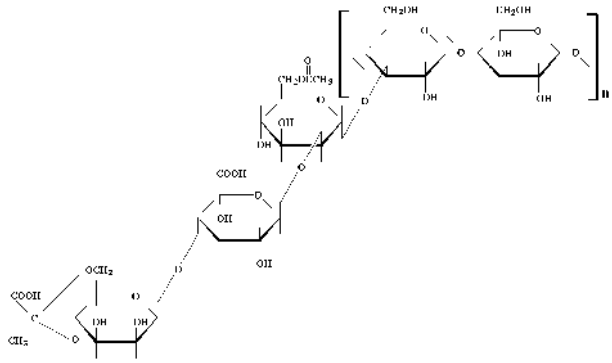

Fig. 1. Structural unit of xanthan gum.

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Xanthan gum in aqueous solutions exhibits three desirable properties: (I) high viscosity at low concentrations; (II) pseudoplasticity; and (III) insensitivity to a wide range of temperature, pH, and electrolyte concentrations. Due to its special rheological properties, the biopolymer is widely used in food, cosmetics, pharmaceuticals, paper, paint, textiles, and adhesives and otherwise in the oil and gas industry [10, 13].

However, because of its high production costs, xanthan loses some markets to polymers from algae or plants [1, 14]. In order to reduce production costs and to improve the competitive position of xanthan, its productivity should be increased [10, 14]. Various approaches have been suggested for this purpose, including (I) development of more efficient fermentation processes [15]; (II) optimization of culture media used for the gum production [15, 16]; and (III) isolation of *X. campestris* mutants with enhanced xanthan production [17-19].

The aim of the present study is to describe mutagenesis of *X. campestris* PTCC 1473 and isolation of xanthan hyperproducing strains. The mutants were further characterized for possible alterations in phenotypic properties such as extracellular enzyme production, consuming different carbon sources, and pigmentation. Pyruvyl and acetyl contents, rheological properties and average molecular weight of the different xanthan gums synthesized by the mutants were also analyzed.

MATERIALS AND METHODS

Materials. Ethyl methanesulfonate (EMS) was purchased from Sigma Chemical Co. (USA) and antifoam A emulsion from Fluka (Switzerland). Rifampicin and all media bases were supplied by Merck Co. (Germany). All other chemicals were of analytical grade.

Microorganism. *X. campestris* PTCC 1473 (NRRL B-1459) was obtained from Persian Type Culture Collection, Iranian Research Organization for Science and Technology (Tehran, Iran) and used as the parent strain. The stock cultures were maintained on yeast malt (YM) agar slants containing (g/l) glucose, 10; peptone, 5; yeast extract, 3; malt extract, 3; and agar, 20, and stored at 4°C. They were transferred every 14 days to maintain good viability and stability for xanthan production.

Batch culture conditions. The culture medium used for xanthan production was the one optimized elsewhere [16]. This culture medium had the following composition (g/l): glucose, 30; $MgSO_4.7H_2O$, 0.24; $(NH_4)_2SO_4$, 3.33; H_3BO_3, 0.0072; $FeCl_3.6H_2O$, 0.0042; KH_2PO_4, 7.2; $CaCO_3$, 0.029; citric acid, 2; ZnO, 0.006; yeast extract, 0.75; peptone, 0.34; antifoam, 0.06; and HCl, 0.16 ml/l. The initial pH of the medium was adjusted to 7.0 ± 0.1. Batch fermentations were carried out in 500-ml Erlenmeyer flasks containing 100 ml of culture medium inoculated with 10% (v/v) of a 24-h preculture. The cultures were then incubated at 30°C on a rotary shaker at 200 rpm for 96 h. Samples were withdrawn at regular intervals and analyzed for concentrations of biomass, xanthan, and residual glucose.

Mutagenesis. The technique for EMS treatment was based on that described by Miller [20] with some modifications. *X. campestris* PTCC 1473 was grown at 30°C in 100 ml YM broth to ca. 2×10^8 cells/ml. Portions (5 ml) of the culture were centrifuged at 5,000 ×g for 15 min, washed twice in minimal A buffer (g/l: K_2HPO_4, 10.5; KH_2PO_4, 4.5; $(NH_4)SO_4$, 1 and sodium citrate.$2H_2O$, 0.5; pH 7.0 ± 0.1) [20], and resuspended in 2.5 ml of the same buffer. The cell suspensions were pooled in a flask and subsequently dispensed as 2-ml aliquots into screw-capped tubes. Then 40 µl EMS was added to each tube, and the tubes were incubated at 30°C with mild shaking for 5 to 60 min. To quench the mutagen, 4 ml of a freshly prepared 10% (w/v) sodium thiosulfate sterile solution was added to each tube [21]. The treated cells were then centrifuged (5,000 ×g for 15 min), washed twice in minimal A buffer and finally resuspended in 2 ml of the same buffer. A 0.5-ml aliquot of each suspension was transferred into 10 ml YM broth and incubated at 30°C overnight to allow mutations to segregate. The day after, suitable dilutions of the cultures were made in sterile 0.9% (w/v) NaCl and bacteria were spread over the surface of YM agar containing rifampicin (50 µg/ml) and incubated at 25-30°C for 72 h [19]. The isolated mutants were further characterized by transferring onto plates of (I) minimal medium (g/l: glucose, 5; K_2HPO_4, 10.5; KH_2PO_4, 4.5; $(NH_4)SO_4$, 1; sodium citrate.$2H_2O$, 0.5; $MgSO_4.7H_2O$, 0.246; thiamine hydrochloride, 0.005 and agar, 15; pH 7.0 ± 0.1) [20]; (II) minimal medium supplemented with casein hydrolysate (acid hydrolyzed) [22]; (III) M9 medium plus glucose, mannose, fructose or lactose at 1% (w/v) as the carbon source [19]; and (IV) YM agar

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containing 1% (w/v) soluble starch, skim milk or sodium carboxymethyl cellulose. The use of these media permitted detection of (i) auxotrophs in general; (ii) amino acid-requiring auxotrophs; (iii) ability of mutants in utilizing different carbon sources; and (iv) extracellular enzymatic activities of α-amylase, protease and cellulase, respectively. The plates were then incubated at 25-30°C for appropriate periods of time. Cleared zones appearing around colonies with protease activity were readily observed on plates containing skim milk. Starch plates were fumigated with iodine to develop halos. To visualize cleared zones produced by cellulase activity, the plates were treated using Congo red as previously described [23].

Absorption spectra of xanthomonadin pigments. Extraction of xanthomonadin pigments was carried out as previously described with some modifications [24]. Overnight cultures of the parent strain and the mutants grown in YM broth were harvested by centrifugation (10,000 ×g for 20 min) in 80-ml polypropylene centrifuge tubes and washed twice in distilled water. After adding methanol at a ratio of 80 ml to 1 g of wet biomass, the tubes were immersed in a boiling water bath for 5 min. After removing cells by centrifugation, the supernatants were concentrated by evaporation in a hot water bath (75°C) to give appropriate concentrations for scanning the absorption spectra. The extracts were scanned using a CECIL CE 9260 spectrophotometer (Cecil Instruments Ltd., England).

Analytical methods. For biomass estimation, cells were pelleted by centrifugation (20,000 ×g for 1 h), washed twice and resuspended in distilled water and absorbance was measured at 650 nm. The corresponding dry weight of the cells was obtained from the established standard curve in which absorbance was plotted against dry biomass [14]. Xanthan was removed from the fermentation medium by precipitation with three volumes ethanol in the presence of 1% (w/v) KCl as the electrolyte [25]. The precipitate was then dried to constant weight at 50°C. Glucose concentration in the cell free supernatants was determined using the glucose oxidase method [26]. The pyruvate content of the polymer was estimated by reaction with 2,4-dinitrophenylhydrazine as described elsewhere [27]. The acetyl content was determined by hydroxamic acid reaction using glucose pentaacetate as the standard [28].

Rheological studies were carried out using a Brookfield DV-II cone and plate viscometer, equipped with a CP-40 spindle. Viscosities of 3 g/l xanthan solutions in 0.01 M KCl were measured at 25°C between shear rates of 2.25 and 225 s^{-1}. The viscosity coefficient (η´) and flow behavior index (N) were derived by linear regression using the following power law model:

$$F^N = \eta' G \qquad (1)$$

where F is the shear stress and G is the shear rate [29, 30].

The average molecular weight of xanthan was obtained by measuring the viscosities of xanthan solutions in 0.1 M KCl at concentrations between 1 and 1.5 g/l. The temperature was maintained at 25°C using a circulatory water bath. The value of the intrinsic viscosity ([η]) was determined and related to the polymer average molecular weight (M_W) by means of a Mark-Houwink type equation proposed by Milas *et al.* [31]:

$$[\eta] = 1.7\times10^{-7} M_W^{1.14} \qquad (2)$$

Statistical analysis. Comparisons between the groups were made with the Dunnett t-test (2-sided) following one-way analysis of variance (ANOVA) treating the parent strain as the control group. In terms of glucose consumption rate, Mann-Whitney U test was used to compare each mutant against the parent strain. Differences between experimental groups were considered significant at the 0.05 level. All analyses were performed using the statistical software SPSS® 10.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Isolation and characterization of mutants. Exposing *X. campestris* PTCC 1473 to EMS generated a collection of mutants. Of 4400 colonies appeared on the rifampicin-containing media, 22 colonies were selected according to their size, color or morphology. These isolates were then checked for their xanthan producing ability in the fermentation medium. They showed xanthan yields between 8.07 and 17.30 g/l compared to the 13.31 g/l obtained from the parent strain (data not shown). Four mutants designated as $XC1473E_1$ to $XC1473E_4$ were observed to accumulate higher amounts of xanthan with respect to the parent strain. Experimentation with these mutant strains was repeated for a one-year period, revealing no

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Table 1. Biochemical characteristics of *X. campestris* PTCC 1473 and the mutants[a].

Strain	MM	MM+CH	M9 medium				Extracellular enzymatic activity		
			+G	+F	+M	+L	A	P	C
Parent	+	+	+	+	+	+/−	+	+	+
$XC1473E_1$	+	+	+	+	+	+/−	+	+	+
$XC1473E_2$	+	+	+	+	+	+	+	+	+
$XC1473E_3$	+	+	+	+	+	+	+	+	+
$XC1473E_4$	+	+	+	+	+	+	+	+	+

[a]Symbols: MM (Minimal medium); CH (Casein hydrolysate); G (Glucose); F (Fructose); M (Mannose); L (Lactose); A (α-Amylase); P (Protease); C (Cellulase); + (Growth); +/− (Faint growth).

alterations in stability or performance. Mutant $XC1473E_1$ appeared yellow on YM agar, the same as the parent strain, but mutants $XC1473E_2$, $XC1473E_3$ and $XC1473E_4$ gave rise to white colonies on the medium. Characterization studies of the strains demonstrated that the mutants were similar to the parent strain except for lactose exploitation as the only carbon source (Table 1). In lactose-containing medium, the parent strain and $XC1473E_1$ grew weakly opposing to the three other mutants. Of these three mutants, $XC1473E_2$ gave a distinctively larger colony in this medium.

Batch fermentation kinetics. Time course fermentation studies were performed for the strains with glucose (30 g/l) as the carbon source to compare the xanthan production and other fermentation parameters of the mutants with the parent strain. The specific growth rate (μ) was determined from the slope of the semi logarithmic plot of cell density versus fermentation time. After 48 h, cultures entered the stationary phase and the rate was calculated in the exponential growth phase. As shown in Table 2, the specific growth rate of $XC1473E_1$ (0.059 h^{-1}) was significantly larger than that of the parent strain (0.054 h^{-1}), resulting in a markedly higher average cell density (reached at the stationary phase) for $XC1473E_1$ compared to the parent strain (4.46 vs. 3.44 g/l). The xanthan formation rate was estimated from the slope of the xanthan production curve of the batch culture. Since the curve almost leveled off after 72 h, the xanthan formation rate was only calculated up to the time. Mutants $XC1473E_2$ and $XC1473E_3$ had higher xanthan formation rates (0.23 and 0.20 g/l.h, respectively) with respect to the parent strain (0.16 g/l.h) (Table 2). The glucose consumption rate was determined from the slope of the glucose consumption curve of the batch culture. As shown in Table 2, mutants $XC1473E_2$ and $XC1473E_3$ exhibited considerably higher glucose consumption rates (0.39 and 0.38 g/l.h, respectively) than the parent strain (0.35 g/l.h), which may be attributed to better xanthan formation rates of the two strains. Mutant $XC1473E_2$ was the only strain to use up glucose within 72 h.

Mutants $XC1473E_2$ and $XC1473E_3$ showed the highest xanthan yield (17.30 g/l) and (16.18 g/l), respectively when compared to the parent strain (13.31 g/l) (Table 2). The yield increases observed for $XC1473E_2$ and $XC1473E_3$ were better than those previously reported by Harding *et al.* [32] and Thorne *et al.* [33] that were 10% and 20%, respectively. However, in more suitable fermentation conditions (e.g. in a fermentor) the yields may be better, because broth viscosity arising from the xanthan production significantly reduces the mass transfer rate for oxygen and other nutrients [34]. Since synthesis of xanthan by *X. campestris* is a very efficient process [34] only relatively small

Table 2. Fermentation kinetic parameters of *X. campestris* PTCC 1473 and the mutants[a,b].

Strain	μ (h^{-1})	R_P (g/l.h)	R_S (g/l.h)	X_S (g/l)	Xanthan[c] (g/l)
Parent	0.054 ± 0.001	0.16 ± 0.01	0.35 ± 0.00	3.44 ± 0.82	13.31 ± 0.46
$XC1473E_1$	0.059 ± 0.002[d]	0.17 ± 0.03	0.35 ± 0.03	4.46 ± 0.49[d]	14.08 ± 1.37
$XC1473E_2$	0.054 ± 0.002	0.23 ± 0.01[d]	0.39 ± 0.03[e]	3.23 ± 0.28	17.30 ± 0.81[d]
$XC1473E_3$	0.055 ± 0.002	0.20 ± 0.01[d]	0.38 ± 0.01[e]	3.54 ± 0.87	16.18 ± 1.12[d]
$XC1473E_4$	0.057 ± 0.001	0.18 ± 0.01	0.36 ± 0.01	3.69 ± 0.68	14.49 ± 1.61

[a]Symbols: μ (Specific growth rare during 0-48 h); R_P (Xanthan formation rate during 0-72 h); R_S (Glucose consumption rate during 0-72 h); X_S (Average dry biomass in the stationary phase). [b]Data are the means of three independent experiments ± standard deviation. [c]Maximal xanthan is given here. [d]Significant difference from the parent strain (Dunnett t-test, $P<0.05$). [e]Significant difference from the parent strain (Mann-Whitney U test, $P<0.05$).

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Table 3. Characteristics of xanthan gums produced by *X. campestris* PTCC 1473 and the mutants[a,b].

Strain	η´ (mPa.s)	N	Pyruvyl (%)	Acetyl (%)	M_W (Da)
Parent	16.87 ± 3.62	1.22 ± 0.02	7.17 ± 0.21	3.58 ± 0.05	946908 ± 11862
$XC1473E_1$	15.44 ± 1.70	1.22 ± 0.01	6.82 ± 0.55	3.56 ± 0.08	988771 ± 57090
$XC1473E_2$	26.47 ± 4.89[c]	1.27 ± 0.02[c]	7.85 ± 0.29	3.51 ± 0.03	1182634 ± 61627[c]
$XC1473E_3$	24.08 ± 4.36	1.25 ± 0.03	7.92 ± 0.42	3.38 ± 0.26	1078048 ± 47827[c]
$XC1473E_4$	18.94 ± 1.37	1.22 ± 0.01	6.62 ± 0.51	3.56 ± 0.01	1057787 ± 40802

[a]Symbols: η´(Viscosity coefficient); N (Flow behavior index); M_W (Average molecular weight). [b]Data are the means of three independent experiments ± standard deviation. [c]Significant difference from the parent strain (Dunnett t-test, $P<0.05$).

increases in yield are to be expected by strain development. Thus, an enhancement of the productivity by 30% (as shown in this study) is significant for the commercial production of xanthan.

Rheological properties. After resuspending dried potassium salt of xanthan (3 g/l) in 10 mM KCl, the effect of the shear rate on the viscosity of each product was determined and the viscosity coefficient and flow behavior index were derived by linear regression of the observed results. The regression coefficients were all greater than 0.999, indicating a very good correlation. According to equation (1), the higher the flow behavior index the greater the pseudoplasticity of system [30].

As shown in Table 3, maximum values of viscosity coefficient and flow behavior index were observed for mutant $XC1473E_2$ (26.47 mPa.s and 1.27, respectively), which were significantly greater than the parent strain. Even though the values obtained with mutant $XC1473E_3$ were reasonably high, they were not significantly different from the parent strain. Xanthan solutions show pseudoplastic behavior, i.e. apparent viscosity decreases as shear rate increases; no hysteresis loop is evident and shear thinning and structural recovery are instantaneous. Due to these features, xanthan is extensively used as thickener and stabilizer in emulsions as well as in paints [13, 15]. In fact, a higher pseudoplasticity index, as observed with $XC1473E_2$, is counted as an advantage for xanthan solutions. The other important characteristic, which reflects on the polymer quality, is the viscosity coefficient. Xanthan produced by $XC1473E_2$ gives a more viscous solution than that of the parent strain.

Rheological properties of xanthan solutions change with polymer nature, i.e. pyruvate [25] and acetate contents as well as average molecular weight [10, 35]. Some researchers have considered the pyruvate content as the best indicator of the polysaccharide quality; a polymer with a pyruvic acid content of 4% or more is defined as a quality gum [25]. This effect appears to be primarily associated with a promotion of the aggregated form of xanthan by pyruvyl residues, and thereby enhances pseudoplasticity and viscosity of the gum solutions [10]. Inversely, acetate has a stabilizing effect upon the xanthan ordered conformation and leads to a decreased gum viscosity [35, 36]. As shown in Table 3, the pyruvate and acetate contents of the xanthan gums produced by the mutants were not significantly different from that obtained from the parent strain, suggesting that the changed rheological characteristics were attributable to the third factor. Actually, the higher molecular weight of xanthans produced by $XC1473E_2$ (1,183 kDa) and $XC1473E_3$ (1,078 kDa) compared to that of the parent strain (947 kDa) (Table 3) seems to account for its increased viscosity coefficient and flow behavior index, since molecular weight of the polymer has a direct influence on its rheological behaviors [6, 10].

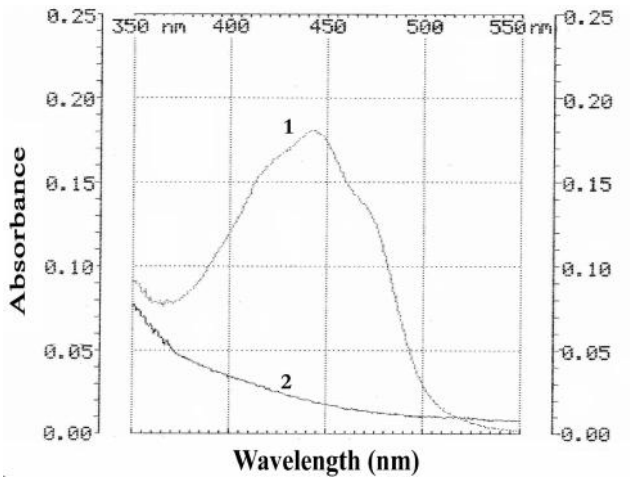

Fig. 2. Absorption spectra of the methanol extracts of *X. campestris* PTCC 1473 (**1**) and mutant $XC1473E_2$ (**2**).

Pigmentation. Colonies of the genus *Xanthomonas* are yellow in color due to the

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production of membrane-bound brominated aryl polyene pigments called Xanthomonadins [37]. Since such pigmentation is unique to this genus, it is commonly used as a marker for chemotaxonomy [37]. Although presence of yellow pigment is an important characteristic for identification, its absence does not exclude an organism from the genus if other characteristics are in agreement [38]. Xanthomonadins from *X. campestris* show three absorption peaks at 418 (a shoulder), 437 and 463 nm when dissolve in petroleum ether [38]. As previously mentioned, mutants XC1473E_2, XC1473E_3 and XC1473E_4 were white-colored contrary to the other two strains; thus for further study, they underwent pigment extraction. As depicted in Figure. 2, the extract obtained from the parent strain showed a peak at 443 nm, but that of XC1473E_2 lacked this absorption maximum, i.e. the wavelength related to yellow pigment. The spectrum of XC1473E_1 extract was the same as that of the parent strain, and those of mutants XC1473E_3 and XC1473E_4 were similar to XC1473E_2 (spectra not shown). Strain selection for production of polysaccharides such as xanthan, obtaining stable strains with high polysaccharide yields and removing unwanted microbial products such as pigment are of special importance in industrial microbiology [39].Xanthan-containing fermentation broths go through some processes such as pasteurization and/or enzymatic treatments, in which microbial cells are lyzed, leading to the release of pigments [38-40]. Therefore, a *X. campestris* strain with no pigmentation, as those isolated in this study, produces xanthan devoiding of color, an organoleptic property that is important for industries like food, cosmetics and pharmaceutical [39].

In conclusion, a number of mutants were derived from *X. campestris* PTCC 1473 using chemical mutagenesis. Of these isolates, mutant XC1473E_2 appeared to be a more favorable xanthan producing strain, because of enhanced polysaccharide yield, higher production rate, enhanced viscosity and pseudoplasticity and lack of yellow pigment. It seems multiple mutations or a pleiotropic mutation affected xanthan biosynthesis pathways and pigmentation at the same time. Isolation of mutants with altered pigmentation has already been reported by other researchers, but those mutants were all defective in xanthan production [24, 32, 41]. Katzen *et al.* [42] isolated mutants with different xanthan biosyntheses but with the same color. Therefore, this study appears to be the first to report isolation of a mutant with enhanced xanthan production without yellow pigmentation and suggests that xanthan synthesis and yellow pigmentation are not necessarily concurrent.

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REFERENCES

1. Lopez, M.J. and Ramos-Cormenzana, A. (1996) Xanthan production from olive-mill wastewaters. *Int. Biodet. Biodegr. 38: 263-270*.
2. Williams, P.H. (1980) Black rot: a continuing threat to world crucifers. *Plant Dis. 64: 736-742*.
3. Jansson, P.E., Keene, L. and Lindberg, B. (1975) Structure of the extracellular polysaccharide from *Xanthomonas campestris*. *Carbohydr. Res. 45: 275-282*.
4. Sandford, P.A., Pittsley, J.E., Knutson, C.A., Watson, P.R., Cadmus, M.C. and Jeanes, A. (1977) Variation in *Xanthomonas campestris* NRRL B-1459: characterization of xanthan products of differing pyruvic acid content. In: *Extracellular microbial polysaccharides.* (Sanford, P.A. and Laskin, A. eds.), American Chemical Society, Washington D.C., USA. pp. 192-210.
5. Sutherland, I.W. (1981) *Xanthomonas* polysaccharides: improved methods for their comparison. *Carbohydr. Polym. 1: 107-115*.
6. Tait, M.I., Sutherland, I.W. and Clarke-Sturman, A.J. (1986) Effect of growth conditions on the production, composition and viscosity of *Xanthomonas campestris* exopolysaccharide. *J. Gen. Microbiol. 132: 1483-1492*.
7. Cadmus, M.C., Rogovin, S.P., Burton, K.A., Pittsley, J.E., Knutson, C.A. and Jeanes, A. (1976) Colonial variation in *Xanthomonas campestris* NRRL B-1459 and characterization of the polysaccharide from a variant strain. *Can. J. Microbiol. 22: 942-948*.
8. Hassler, R.A. and Doherty, D.H. (1990) Genetic engineering of polysaccharide structure: production of variants of xanthan gum in *Xanthomonas campestris*. *Biotechnol. Prog. 6: 182-187*.
9. Sutherland, I.W. (1996) Microbial biopolymers from agricultural products: production and potential. *Int. Biodet. Biodegr. 38: 249-261*.
10. Margaritis, A. and Pace, G.W. (1985) Microbial polysaccharides. In: *Comprehensive biotechnology. The practice of biotechnology: Current commodity products.* (Blanch, H.W., Drew, S. and Wang, D.I.C.

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[DOR: 20.1001.1.1028852.2003.7.3.1.3]

eds.), Vol. 3, Pergamon Press, New York, USA. pp. 1005-1044.

11. Moreira, A.S., Vendruscolo, J.L.S., Gil-Turnes, C. and Vendruscolo, C.T. (2001) Screening among 18 novel strains of *Xanthomonas campestris* pv. pruni. *Food Hydrocoll. 15: 469-474*.
12. Lo, Y.M., Yang, S.T. and Min, D.B. (1997) Effects of yeast extract and glucose on xanthan production and cell growth in batch culture of *Xanthomonas campestris*. *Appl. Microbiol. Biotechnol. 47: 689-694*.
13. Sutherland, I.W. (1998) Novel and established applications of microbial polysaccharides. *Trend Biotechnol. 16: 41-46*.
14. Shu, C.H. and Yang, S.T. (1990) Effects of temperature on cell growth and xanthan production in batch cultures of *Xanthomonas campestris*. *Biotechnol. Bioeng. 35: 454-468*.
15. García-Ochoa, F., Santos, V.E., Casas, J.A. and Gómez, E. (2000) Xanthan gum: production, recovery, and properties. *Biotechnol. Adv. 18: 549-579*.
16. Roseiro, J.C., Esgalhado, M.E., Amaral Collaço, M.T. and Emery, A.N. (1992) Medium development for xanthan production. *Process Biochem. 27: 167-175*.
17. Rodríguez, H. and Aguilar, L. (1997) Detection of *Xanthomonas campestris* mutants with increased xanthan production. *J. Ind. Microbiol. Biotechnol. 18: 232-234*.
18. Marquet, M., Mikolajczak, M., Thorne, L. and Pollock, T.J. (1989) Improved strains for production of xanthan gum by fermentation of *Xanthomonas campestris*. *J. Ind. Microbiol. 4: 55-64*.
19. Pollock, T.J. and Thorne, L. (1995) Biologically pure culture of a strain of *Xanthomonas campestris* ATCC 55429. *US Pat. 5,472,870*.
20. Miller, J.H. (1992) A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, New York, USA.
21. Lawrence, C.W. (1991) Classical mutagenesis techniques. In: *Methods in Enzymology.* (Guthrie, C. and Fink, G.R. eds.), Vol. 194, Academic Press, San Diego, USA. pp. 273-281.
22. Daniels, M.J., Barber, C.E., Turner, P.C., Cleary, W.G. and Sawczyc, M.K. (1984) Isolation of mutants of *Xanthomonas campestris* pv. campestris showing altered pathogenicity. *J. Gen. Microbiol. 130: 2447-2455*.
23. Teather, R.M. and Wood, P.J. (1982) Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Appl. Environ. Microbiol. 43: 777-780*.
24. Chou, F.L., Chou, H.C., Lin, Y.S., Yang, B.Y., Lin, N.T., Weng, S.F. and Tseng, Y.H. (1997) The *Xanthomonas campestris gumD* gene required for synthesis of xanthan gum is involved in normal pigmentation and virulence in causing black rot. *Biochem. Biophys. Res. Commun. 233: 265-269*.
25. Cadmus, M.C., Knutson, C.A., Lagoda, A.A., Pittsley, J.E. and Burton, K.A. (1978) Synthetic media for production of quality xanthan gum in 20 liter fermentors. *Biotechnol. Bioeng. 20: 1003-1014*.
26. Barham, D. and Trinder, P. (1972) An improved color reagent for the determination of blood glucose by the oxidase system. *Analyst 97: 142-145*.
27. Sloneker, J.H. and Orentas, D.G. (1962) Pyruvic acid, a unique component of an exocellular bacterial polysaccharide. *Nature 194: 478-479*.
28. McComb, E.A. and McCready, R.M. (1957) Determination of acetyl in pectin and in acetylated carbohydrate polymers. Hydroxamic acid reaction. *Anal. Chem. 29: 819-821*.
29. Torres, L.G., Brito, E., Galindo, E. and Choplin, L. (1993) Viscous behavior of xanthan aqueous solutions from a variant strain of *Xanthomonas campestris*. *J. Ferment. Bioeng. 75, 58-64*.
30. Martin, A. (1993) Physical pharmacy. 4th ed., Lea and Febiger, Philadelphia, USA.
31. Milas, M., Rinaudo, M. and Tinland, B. (1985) The viscosity dependence on concentration, molecular weight and shear rate of xanthan solutions. *Polymer Bull. 14: 157-164*.
32. Harding, N.E., Cleary, J.M., Cabanas, D.K., Rosen, I.G. and Kang, K.S. (1987) Genetic and physical analyses of a cluster of genes essential for xanthan gum biosynthesis in *Xanthomonas campestris*. *J. Bacteriol. 169: 2854-2861*.
33. Thorne, L., Tansey, L. and Pollock, T.J. (1987) Clustering of mutations blocking synthesis of xanthan gum by *Xanthomonas campestris*. *J. Bacteriol. 169: 3593-3600*.
34. Moraine, R.A. and Rogovin, P. (1966) Kinetics of polysaccharide B-1459 fermentation. *Biotechnol. Bioeng. 8: 511-524*.
35. Dea, I.C.M. (1987) The role of structural modifications in controlling polysaccharides functionality. In: *Industrial polysaccharides: Genetic engineering, structure/property relations and applications.* (Yalpani, M. ed.), Vol. 3, Elsevier, Amsterdam, The Netherlands. pp. 207-216.
36. Martin, M.O. (1994) Synthesis of commercially valuable bacterial polymers: impact of molecular genetics. *Res. Microbiol. 145: 93-97*.
37. Starr, M.P., Jenkins, C.L., Bussey, L.B. and Andrewes, A.G. (1977) Chemotaxonomic significance of the xanthomonadins, novel brominated aryl-polyene pigments produced by bacteria of the genus *Xanthomonas*. *Arch. Microbiol. 113: 1-9*.
38. Krieg, N.R. and Holt, J.G. (1984) Bergey's manual of systematic bacteriology. Vol. 1, Williams and Wilkins, Baltimore, USA.
39. Sutherland, I.W. (1990) Biotechnology of microbial exopolysaccharides. Cambridge University Press, Cambridge, UK.

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40. Becker, A., Katzen, F., Pühler, A. and Ielpi, L. (1998) Xanthan gum biosynthesis and application: a biochemical /genetic perspective. *Appl. Microbiol. Biotechnol. 50: 145-152*.
41. Tseng, Y.H., Choy, K.T., Hung, C.H., Lin, N.T., Liu, J.Y., Lou, C.H., Yang, B.Y., Wen, F.S., Weng, S.F. and Wu, J.R. (1999) Chromosome map of *Xanthomonas campestris* pv. campestris 17 with locations of genes involved in xanthan gum synthesis and yellow pigmentation. *J. Bacteriol. 181: 117-125*.
42. Katzen, F., Ferreiro, D.U., Oddo, C.G., Ielmini, M.V., Becker, A., Pühler, A. and Ielpi, L. (1998) *Xanthomonas campestris* pv. campestris gum mutants: effects on xanthan biosynthesis and plant virulence. *J. Bacteriol. 180: 1607-1617*.

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