

## PRODUCTION AND PHYSICOCHEMICAL CHARACTERIZATION OF XANTHAN GUM BY NATIVE LACTOSE CONSUMING ISOLATES OF *Xanthomonas citri* subsp. *citri*

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*Xanthan* is a biopolymer produced by *Xanthomonas* bacteria which is widely used in many industries such as food and oil. In this work, three *Xanthomonas* strains (*X. citri*/NIGEB-88, *X. citri*/NIGEB-386 and *X. citri*/NIGEB-K37) were used to evaluate their industrial potential to produce xanthan gum in whey medium. Bacteria growth rate, viscosity, biomass, dry weight of produced xanthan and  $\beta$ -galactosidase activity were studied during the fermentation process and the presence of  $\beta$ -galactosidase genes was assessed by PCR technique. Strain NIGEB-386 had the best ability to utilize lactose in the whey medium. The highest amount of xanthan production and viscosity were 22.7 g/l and 2066.6 mPa·s, respectively. The presence of six  $\beta$ -galactosidase genes in strains NIGEB-386 and NIGEB-K37 was confirmed. The pyruvate and acetyl contents in xanthan gum were 2.1 and 0.29 %, respectively. Fourier-transform infrared spectroscopy analysis determined the position of the functional groups in the structure of the fermentation product. In whey medium, the performance of both NIGEB-386 and NIGEB-K37 strains were better than the *X. campestris*. The findings showed that *Xanthomonas citri*/NIGEB-386 is suitable for industrial production of xanthan using cheese whey as a low-cost medium.

**Key words:** *Xanthomonas citri*, Xanthan gum, cheese whey,  $\beta$ -galactosidase, pyruvate and acetate content, FTIR.

The genus of *Xanthomonas* comprises different species that cause multiple diseases in a wide variety of plant species. Bacterial citrus canker is one of the most destructive citrus diseases caused by *Xanthomonas citri* subsp. *citri* (*Xci*) [1]. This bacterium is able to produce water-soluble exopolysaccharides that can sustain bacteria in poorly maintained environments. This polymer, which is also called xanthan, plays a role in biofilm formation and bacterial pathogenicity [2]. Xanthan has unique properties such as high viscosity, pseudo elasticity and stability at various temperatures, and because of the later, it is widely used in many fields of food, oil and pharmaceutical industries [3]. Xanthan gum is an emulsifier that helps ingredients to blend more

effectively. It is also used as a thickener in many fat-free foods. In pastry fillings, it prevents water from seeping out and soaking the dough [4]. The structure of xanthan gum consists of cellulose like skeleton with different proportions of mannose, glucose, glucuronic acid, pyruvic acid and acetyl groups attached to the side chain, and thus creating different rheological properties in xanthan solutions [5]. Based on the substitution of the acetyl and pyruvic acid units in the side chain of the xanthan structure, its molecular weight varies. Generally, 66-88% and 5-21% of mannose in the side chain of xanthan undergo pyruvation and acetylation, respectively [6]. There are a lot of reports on the use of *Xanthomonas campestris* as a major strain in xanthan production; however, there

are limited ones on using *Xanthomonas citri* species as producer of xanthan gum. The synthesis of xanthan gum is believed to be similar to exopolysaccharide synthesis by other gram negative bacteria. Xanthan is produced through aerobic fermentation at a temperature between 27 °C and 30 °C [22]. In industrial scale, the xanthan gum is normally produced by batch fermentation, thermal treatment, cell removal, recovery with alcohol, drying and milling of the gum [3]. Generally, xanthan is produced from carbon sources such as glucose, sucrose and starch [7]. Sugar concentration in the range of 2-4%, in the culture media, has been reported to be optimal for xanthan production [3]. In the commercial process, the microorganism can convert approx. 70% of substrate to gum [22]. However, due to the relatively high costs of these sugars, the production of xanthan gum is costly [8, 9]. In this case, the use of dairy wastes such as a carbon substrate can be considered as a proposed solution for xanthan production because not only it decreases environmental pollution, but also it reduces the production cost.

Whey contains approximately 4-5% (w/v) lactose, 8-10% (w/v) proteins, as well as small amounts of organic acids, minerals and vitamins [10]. Hence, whey is considered as a highly valuable growth medium for microbial process and it could be chosen as a cheap substrate for xanthan production. Most of *Xanthomonas* wild species do not consume lactose because of their low  $\beta$ -galactosidase activity. To solve this problem, several efforts have been made to transfer the  $\beta$ -galactosidase genes by plasmid or phage vectors to *Xanthomonas* genome and forcing the bacteria into the lactose consumption [11]. The use of mutagenesis methods with mutagenic agents such as Nitric acid, Ethyl Methane sulfonate (EMS), Acridine dye and UV radiation has also been used to develop mutated strains for lactose consumption [12, 13]. But genetic modification is usually associated with some disadvantages, including the production of antibiotic-resistant strains and strain instability [12]. The risk of genetically modified organisms (GMO) in food industries may also be a prohibitive factor. To solve this problem, the best option is to use the native strains of *Xanthomonads* that naturally have the ability to use lactose for xanthan production [14].

According to the recent studies, several new strains of *Xanthomonas citri* have been isolated from citrus plants in the southern part of Iran [15]. Based on these reports, we selected three native

*Xanthomonas citri* subsp. *citri* strains, NIGEB-K37, NIGEB-088 and NIGEB-386, and compared their respective capabilities to produce xanthan in cheese-whey medium. Moreover, we looked for the presence of  $\beta$ -galactosidase genes in these strains, which could play an important role in lactose consumption for xanthan production.

## Materials and Methods

**Microorganism and media.** Three local isolates of *Xanthomonas citri* subsp. *citri*, NIGEB-K37, NIGEB-088 and NIGEB-386, were obtained from NIGEB's Bacterial Citrus Canker Collection. *X. campestris* pv. *campestris* DSMZ1706 (*Xcc*/DSMZ1706) was used as a control. Yeast malt (YM) agar containing 3 g·l<sup>-1</sup> yeast extract, 3 g·l<sup>-1</sup> malt extract, 5 g·l<sup>-1</sup> peptone, 10 g·l<sup>-1</sup> glucose, 20 g·l<sup>-1</sup> agar in distilled water (pH 7.2) was used as a culture medium for *Xci* strains. These bacterial strains were maintained at 30 ± 1 °C on YM agar slants and sub-cultured every 15 days for six months to prevent the strains from losing their production capability [3].

**Preparation of inoculum.** For pre-culture preparation, cells were initially inoculated into the YM agar and incubated at 28 °C for 18-24 hours until colonies were developed on the surface of the medium. Cell propagation was done in two steps. As the first step, one loopful of the cells was transferred into 50 ml of YM broth medium and incubated at 28 °C, 120 rpm, till an optical density of 2 (at 600 nm) was reached. Two ml of this pre-inoculum was then transferred to a 500 ml flask, containing 100 ml of the YM broth, and incubated at 28 °C, 120 rpm for 24 h. Before inoculation of preculture to the production medium, cell concentration reached to about 1×10<sup>8</sup> colony forming units (CFU) ml<sup>-1</sup> [9].

**Cheese-Whey Preparation and Xanthan Production.** The production medium, which was used for xanthan production, was prepared based on cheese whey powder (Pegah Dairy Co. Tehran, Iran) as a carbon source. Ingredients of the whey powder are shown in Table 1. Cheese proteins were precipitated and removed by heat treatment. 60 g of whey powder was dissolved in 1 liter of distilled water and heated for 10 min at 100 °C. After heating, the medium was filtered and the supernatant was centrifuged at 10,000×g for 10 min for removing the precipitate [9]. The production medium was prepared by the addition of the following compounds to 1 liter of treated whey: 2.1 g Citric acid, 3 g KH<sub>2</sub>PO<sub>4</sub>, 0.3 g MgSO<sub>4</sub>, 0.04 g CaCO<sub>3</sub>, 0.014 g H<sub>3</sub>BO<sub>3</sub>, 0.02 g ZnSO<sub>4</sub>,

Table 1. Composition of cheese whey powder

Contents	Values (%)
Fat	1
Protein	14.5
Lactose	67
Ash	8.2
Minerals	8

0.008 g FeCl<sub>3</sub>·6H<sub>2</sub>O, and 2.2 g spray-dried corn steep liquor. Liquid Corn steep liquor (6% dry solids) was procured from Glucosan Co. Ghazvin, Iran, and sprays dried by an APV Compact® Spray Dryer (APV Anhydro AS, Denmark). The pH of the medium was adjusted to 7.15 by adding NaOH before autoclaving [3]. 100 ml of the production medium was inoculated with 10% (v/v) of inoculum from the previous stage and incubated at 28 °C on a rotary shaker at 200 rpm for 5 days. During the fermentation process, sampling was carried out at 24-hour intervals, and β-galactosidase activity, residual sugar, viscosity, and xanthan content were measured [16].

**Measurement of Bacterial Growth.** Bacterial growth was measured spectrophotometrically in 600 nm (UV Spectrophotometer-Unicam 8620). Enumeration of live cells was also done by the plate count method on YM agar [16].

**Determination of Lactose Utilization and β-Galactosidase Activity.** In order to evaluate and compare lactose consumption and β-galactosidase activity of strains during fermentation, sampling was carried out at intervals of 0 to 120 h and each sample was treated with acid hydrolysis method [17]. Then, the amount of sugar was measured by DNS method [18]. The assay of the enzyme activity was performed as described by Miller [18]. One unit of β-galactosidase was defined as the amount of enzyme that produced 1 nmol of ortho-nitrophenol (ONP) per min [10, 18].

**Measurement of Viscosity and Dry Weight of Xanthan.** Analysis of apparent viscosity was carried out to assess the rheological feature of the produced xanthan [16]. The viscosity of the fermentation broth was measured by using BROOK-FIELD DV-II viscometer at 25 °C [15, 19]. To obtain the amount of xanthan produced, the samples were taken at specific time intervals, diluted with distilled water (1:1 v/v), and centrifuged at 10000×g for 15 min at 4 °C for separating the cells. The supernatant was mixed with ethanol (1:3 v/v) [3] to precipitate the polymer. The

biopolymer was separated and dried by freeze-drying (LYOVACTM-GT 3) and weighed [3, 16].

**Detection of β-Galactosidase Genes by PCR.** The Multiplex-PCR method was used to study the presence of β-galactosidase genes. A total of six primer pairs were designed to replicate β-galactosidase genes. The characteristics of designed primers such as GC percentage, temperature T<sub>m</sub>, and the probability of loop formation were individually examined by using of soft-Analyzer Oligo (Developer: Molecular Biology Insights Inc. version: 7, USA). PCR was performed as described by the manufacturer (PEQLAB® Thermocycler) and the amplified genes were evaluated for the expected products on 1.5% agarose gel and photographed on a UV Trans illuminator Ingenious LBR model [11]. The specific primers designed are shown in Table 2.

**Pyruvate and acetate assays.** The colorimetric method was used for the determination of pyruvate and acetate content in xanthan product. The percentage of pyruvate and acetate was measured by 2 and 4 di-nitrophenylhydrazine and hydroxamic acid methods, respectively [16, 19]. The standard acetylcholine and pyruvate curves were prepared and the commercial xanthan powder (Sigma-Aldrich) was used as control.

**Fourier Transform Infrared Spectroscopy (FTIR) Analysis.** Fourier transform infrared spectrum (FTIR) was used to identify the functional groups in the structure of xanthan gum [20, 21]. The infrared spectra of xanthan from the current study and commercial xanthan (from *Xanthomonas campestris*; Sigma-Aldrich; No: 11138-62-2) were compared. The product of xanthan was powdered and mixed with KBr and was prepared as a pellet. Samples were analyzed using Perkin Elmer Fourier Transform Infrared, with wavelength ranging from 400 to 4000 waves/cm with 32 scans/samples [Perkin Elmer, spectrum one] [9, 19].

**Statistical Analysis.** All experiments were replicated in thrice and the data were analyzed by using the SPSS® 12 software. A value of  $P < 0.05$  was considered as statistical significance.

## Results and Discussion

**Cell Growth during Fermentation.** To investigate the growth rate of bacteria in the production medium, optical absorption at 600 nm and colony count were recorded at the time of sampling. As shown in Fig. 1, A, almost all strains reached a logarithmic phase (OD<sub>600</sub> of ≤2) between 16–24 h. Based

Table 2. Primers used in this study

Primers	Sequence	Product size, bp
<i>Xci</i> /β-gal1 F	5' AGATCGAGAACGAATACAGC 3'	343
<i>Xci</i> /β-gal1 R	5' TACAGGTTGAACGAATAGCC 3'	
<i>Xci</i> /β-gal2 F	5' AATCCCAGTGACCACTACGC 3'	465
<i>Xci</i> /β-gal2 R	5' GCTGTTTTTCGACCAGGACAT 3'	
<i>Xci</i> /β-gal3 F	5' TATGGCTCATCTTGCTGCTG 3'	543
<i>Xci</i> /β-gal3 R	5' CGTTGTATCCGCTGTTGATG 3'	
<i>Xci</i> /β-gal4 F	5' GTGCTGGATGTGTGGAAGG 3'	231
<i>Xci</i> /β-gal4 R	5' ATAGCGGGTGTAGTCGATGC 3'	
<i>Xci</i> /β-gal5 F	5' ATGAAACGCAGGAGCCTATG 3'	376
<i>Xci</i> /β-gal5 R	5' GATACACCCACATGCCTTCC 3'	
<i>Xci</i> /β-gal6 F	5' GTTTCGTGAGTGCATCGTGT 3'	164
<i>Xci</i> /β-gal6 R	5' CTTCGTAGAACCAGCCTTCG 3'	

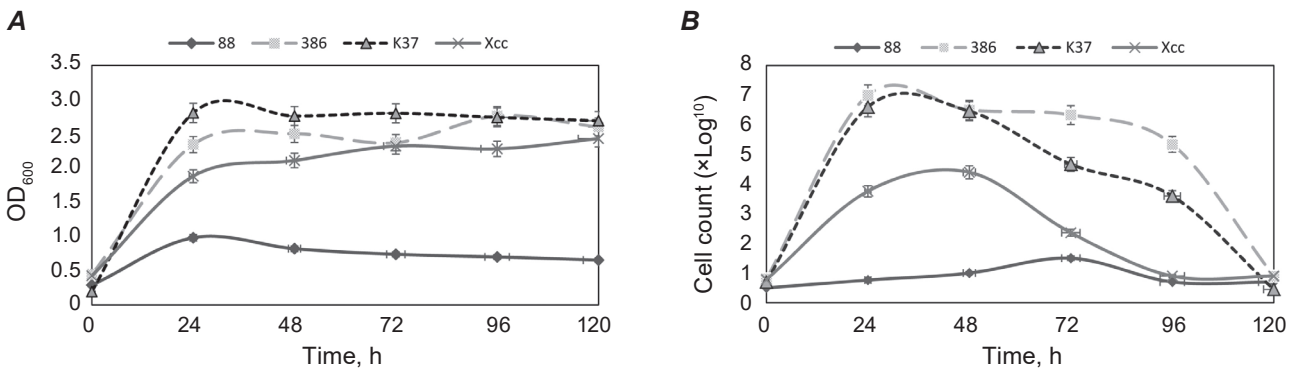


Fig. 1. Comparison of the growth rate of *Xanthomonas* strains in whey medium during fermentation: (A) cell count by optimal density at 600 nm, and (B) cell count by colony counting on YM agar ( $P \leq 0.05$ )

on the data, after around 24 h, the bacterial growth was substantially constant except for the NIGEB-088 strain, which showed poor growth ( $OD_{600} = 1$ ) in the whey medium. The colony count also showed that the number of bacteria decreased sharply after 24 h of the beginning of the process, and then entered a stationary phase (Fig. 1, B).

**Lactose Consumption and β-Galactosidase Activity.** In NIGEB-386 and NIGEB-K37 strains, the amount of lactose sugar was significantly decreased after 24 h (Fig. 2, B). The NIGEB-088 strain did not use lactose sugar. The reduction of lactose in NIGEB-386 and NIGEB-K37 strains and even *Xcc*/DSMZ1706 was consistent with the increase of β-galactosidase enzyme activity. The highest enzyme activity was measured for NIGEB-

K37 and NIGEB-386 strains after 96 h with values of 1259.31 U/ml and 1302.98 U/ml, respectively ( $P \leq 0.05$ ) (Fig. 2, A).

**Xanthan Production and Viscosity.** The results of the culture viscosity of the *X. citri* strains during fermentation are shown in Fig. 3, A. During the first 72 h, there was no change in the viscosity of the culture media, but after 72 h, the viscosity increased exponentially. The strain NIGEB-386 showed the highest viscosity score (2066.6 mPa.s); however, the strain NIGEB-088 did not change the medium viscosity and produce any xanthan. Xanthan gum produced by the other strains was dried and then weighed after precipitation by ethanol. After 48 to 72 h (in the early stationary phase), xanthan production began. At the end of the fermentation pro-

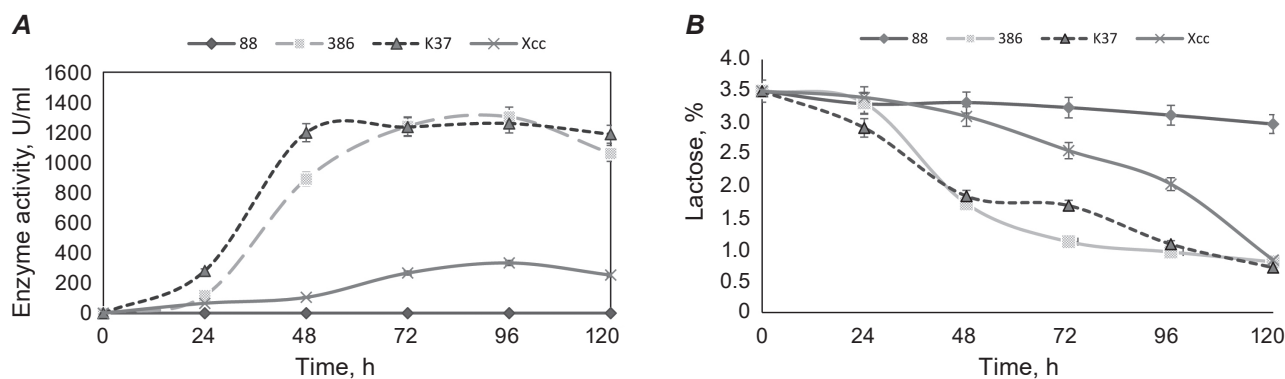


Fig. 2. Comparison of enzyme activity and lactose consumption by studied strains in cheese whey medium. (A)  $\beta$ -galactosidase activity, and (B) lactose consumption by strains ( $P \leq 0.05$ )

cess, the strains NIGEB-386, NIGEB-K37 and *Xcc*/DSMZ1706 produced 20.3, 18.4 and 13.7 g/l of xanthan, respectively ( $P \leq 0.05$ ) (Fig. 3, B).

*$\beta$ -Galactosidase genes of strains.* To understand the relationship between  $\beta$ -galactosidase activity and the presence of related genes, PCR was performed in two strains of NIGEB-K37 and NIGEB-386, which had the highest  $\beta$ -galactosidase activity (Fig. 2). Bands with specific sizes ranging from 164 to 543 bp are related to  $\beta$ gal 1 to 6 genes (Fig. 4, 5). These results confirmed that all 6 genes are involved in elevating the  $\beta$ -galactosidase level in *Xcc*/DSMZ1706 as control and both NIGEB-K37 and NIGEB-386 strains. There were differences in the concentration of some amplified fragments, as well as in the occurrence of numerous non-specific bands (Fig. 5).

*The chemical structure of xanthan.* Comparison of the fermentation results showed that the

strain NIGEB-386 was superior to the NIGEB-K37 regarding the concentration of the final product and viscosity of the culture broth, so it was selected for further studies and structural analysis. Comparison of xanthan viscosity of 1% (w/v) solution, acetate and pyruvate content of the produced polymer by *X. citri*/NIGEB-386 and *Xcc*/DSMZ1706 with commercial xanthan gum (Sigma-Aldrich) is shown in Table 3. FTIR spectra of xanthan gum from strain *Xci*/386 was compared with that of standard xanthan gum (Sigma-Aldrich) for the characterization of functional groups (Fig. 6). The presence of carbonyl, hydroxyl and acetyl groups has been confirmed in xanthan's structure. The most important bands recorded in the range of 4000-400  $\text{cm}^{-1}$  were as follow: 3420-3430  $\text{cm}^{-1}$ : -OH; 2850-2950  $\text{cm}^{-1}$ : C-H and CHO; 1710-1735  $\text{cm}^{-1}$ : C=O ester, acid carboxylic, aldehydes and ketones; 1600-1630  $\text{cm}^{-1}$ : C=O 1400-1415  $\text{cm}^{-1}$ : C-H and 1050-1255  $\text{cm}^{-1}$ : C-O (Table 4).

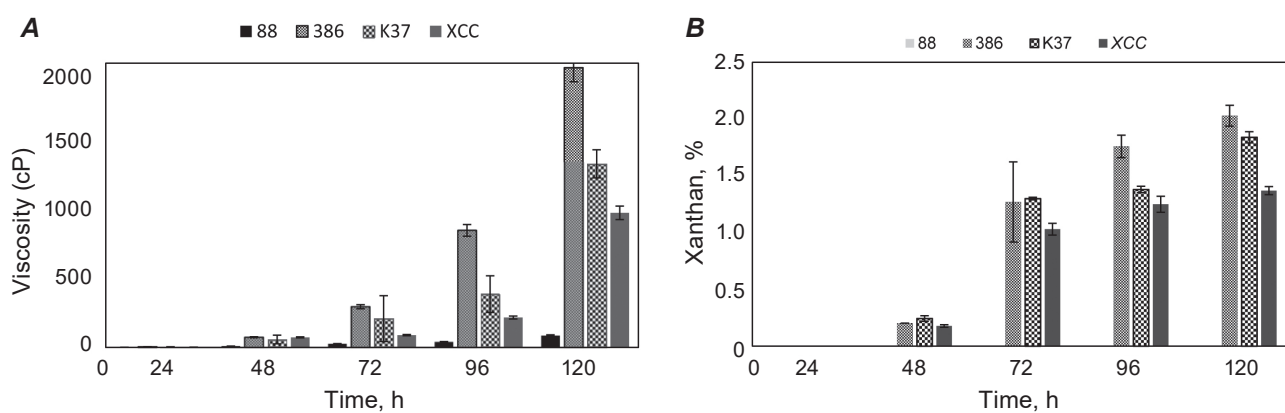


Fig. 3/ Comparison of Xanthan production behavior by studied strains in cheese whey medium (A) Viscosity of the fermentation broth which measured by Ostwald viscometer. (B) Dry weight of xanthan produced (g/l), ( $P \leq 0.05$ )

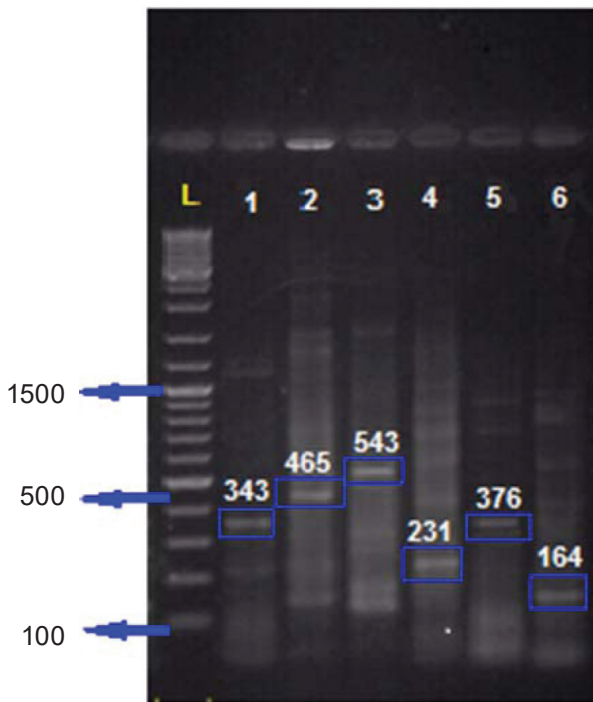


Fig. 4/ Gel electrophoresis of  $\beta$ -galactosidase genes in PCR products of *X. campestris* DSM1706(*Xcc*/DSM1706) as control. Lane L: DNA ladder 100 bp plus, Lane 1-6: PCR fragments with specified size

In spite of minor differences of recorded picks in the FTIR spectra, the correspondence between bands led them to assert a strong similarity between commercial xanthan (Sigma-Aldrich) and produced xanthan by strain NIGEB-386 due to acid groups presents.

Generally, glucose, sucrose and starch are used as the most important sources for xanthan gum production. But the use of cheaper sources of carbon from agricultural and industrial wastes such as molasses and whey can be considered more appropriate for the production of xanthan at the industrial scale [20]. Due to the entry of industrial wastewater into the environment and surface water, the level of BOD would increase, which has gained environmental concerns [22]. One of the strategies for reducing BOD level is the use of whey as a suitable food source for the production of microbial products such as xanthan [16]. Whey is rich in lactose and the use of those *Xanthomonas* strains, which have a natural ability to utilize unconventional sugars such as lactose, will be more economical [15]. *X. campestris* is often used for the production of xanthan. This study evaluated the suitability of native and non-GMO strains of *Xanthomonas citri* for the production of xanthan in whey containing medium. In all the ex-

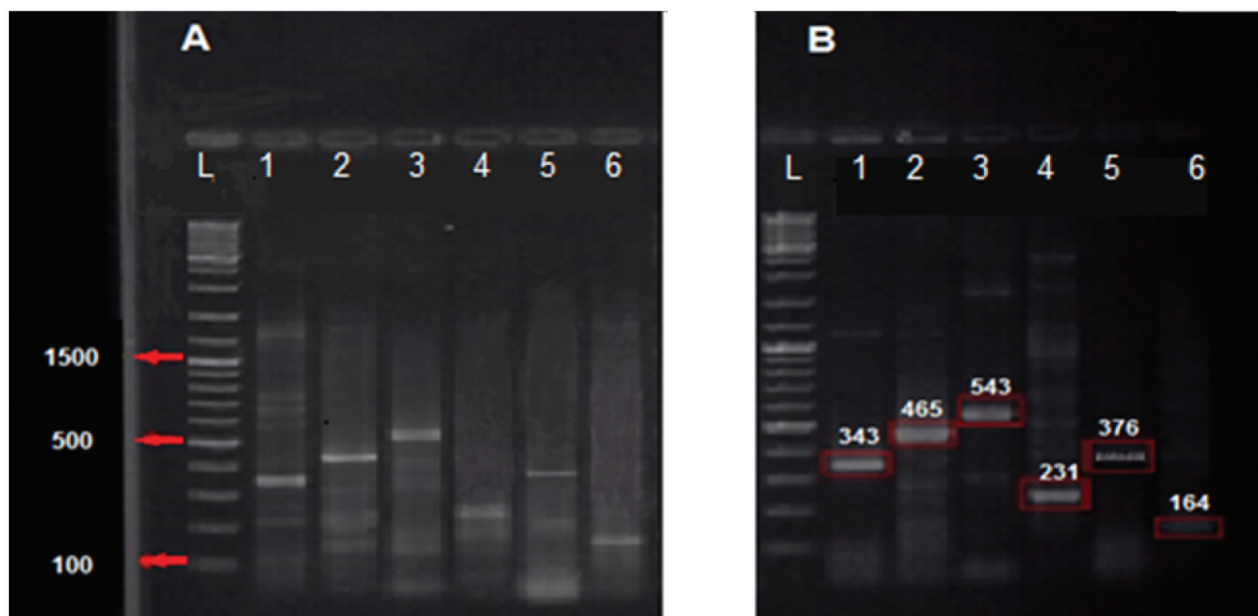


Fig. 5. Gel electrophoresis of  $\beta$ -galactosidase genes in PCR products of local isolated *Xanthomonas citri* strain A)  $\beta$ -galactosidase genes are discriminated by band length in *Xci*/NIGEB-K37. Lane L: indicates DNA ladder 100bp plus. Lane 1-6: include the PCR products. B) Gel electrophoresis of *Xci*/NIGEB-386. Lane L: DNA ladder 100bp plus, Lane 1-6: PCR fragments with specified size

Table 3. The comparison of viscosity and acetate - pyruvate contents in commercial and laboratory made xanthan samples

Acetate (% w/w)	Pyruvate (% w/w)	Viscosity (mPa. s) <sup>a</sup>	Bacteria
<i>X. citri</i> /NIGEB-386	325 ± 5 <sup>b</sup>	2.1 ± 0.1	0.289 ± 0.01
<i>Xcc</i> /DSMZ1706	207 ± 17	1.73 ± 0.05	0.17 ± 0.0
<i>X. campestris</i> <sup>c</sup>	695 ± 12	5.40 ± 0.03	0.33 ± 0.0

<sup>a</sup>Xanthan (1% w/v solution). <sup>b</sup> Mean ± SD ( $n = 5$ ). <sup>c</sup>Commercial xanthan (Sigma-Aldrich®)

Table 4. Spectra of functional groups in standard xanthan and xanthan product of *Xci*/NIGEB-386

Functional groups	Hydroxyl	Carbonyl	Carboxyl	Acetyl
Standard xanthan gum (Sigma-Aldrich®)	3426	1634;1733	1253;1454	1076;2927
Xanthan gum synthesized by <i>Xci</i> //NIGEB-386	3421	1611;1735	1252;1454	1076;2933

periments, the *Xcc*/DSMZ1706 strain was used as a positive control. The  $\beta$ -galactosidase activities of NIGEB-386 and NIGEB-K37 strains were about 16-fold more than that of *Xcc*/DSMZ1706 (1302 U/ml and 1259 U/ml, respectively, vs. 333 U/ml) ( $P \leq 0.05$ ). The comparison of graphs A and B in Fig. 2 suggests that because of higher  $\beta$ -galactosidase activity, the bacteria were able to use lactose and eventually reduce the amount of lactose at the end of the process. On the other hand, strain 88's inability to consume the lactose was consistent with the inactivation of  $\beta$ -galactosidase and the lack of ability for bacterial growth. This enzyme activity level was already enough to support growth and also confirm the ability of the NIGEB-K37 and NIGEB-386 strains to consume lactose in whey [10].

Various strains of *Xanthomonas* species can produce xanthan gum with different carbon sources; however, the highest amount of xanthan gum (about 14 g/l) was produced by the wild type of *X. campestris* from lactose source [5, 23]. Ramezani et al., [15] showed that the highest amount of produced xanthan by the NIGEB-K37 strain in a lactose-based medium (lactose 10%) was 14.26 g/l. In this study, we increased the amount of the product to 18.4 g/l in the cheese whey medium without the addition of more lactose by the NIGEB-K37. Cheese whey has a lot of nutritious sources and is cheaper than other lactose sources. Increased viscosity and xanthan production by the NIGEB-386 and NIGEB-K37 strains corresponded with their  $\beta$ -galactosidase activity. Obviously, in an environment, where only lactose is present, the bacteria should be able to use lactose

to synthesize xanthan. The ability to use lactose requires the presence of  $\beta$ -galactosidase activity in bacteria [24]. Therefore, the absence of xanthan production in the NIGEB-088 strain could be attributed to the lack of  $\beta$ -galactosidase enzyme activity.

The activity and expression of the genes involved in the process of lactose utilization by the bacterial strains associated with citrus canker disease (*X. citri* subsp. *citri* 306, *X. fuscans* subsp. *aurantifolii* and *X. alfalfa* subsp. *citromelonis*) have not been investigated extensively [25]. However, based on the  $\beta$ -galactosidase genes registered in the gene database (NCBI), as well as the use of the ONPG substrate and its relationship with the expression level of the lac operon, the presence of these genes in the native strains is possible [26]. Yang et al., [10] carried out a PCR amplification to detect the  $\beta$ -galactosidase gene in a strain of *Xc17* (*X. campestris* 17). Their results indicated that *Xc17* indeed contained a  $\beta$ -galactosidase gene; however, the enzyme levels were too low to support the growth of the on lactose-containing medium. The PCR results of this study confirmed the presence of  $\beta$ -galactosidase genes in the native strains of NIGEB-386 and NIGEB-K37. Although the sequencing of these genes was not carried out in this study, previous studies have shown that the position of these genes varies on the *Xanthomonas citri* genome and some of them are functional and some are also putative genes [27]. The relatively high activity of  $\beta$ -galactosidase in both strains (NIGEB386 and K37) compared with the commercial strain of *Xcc*/DSMZ1706 may indicate a remarkable expression level of these genes. Howe-

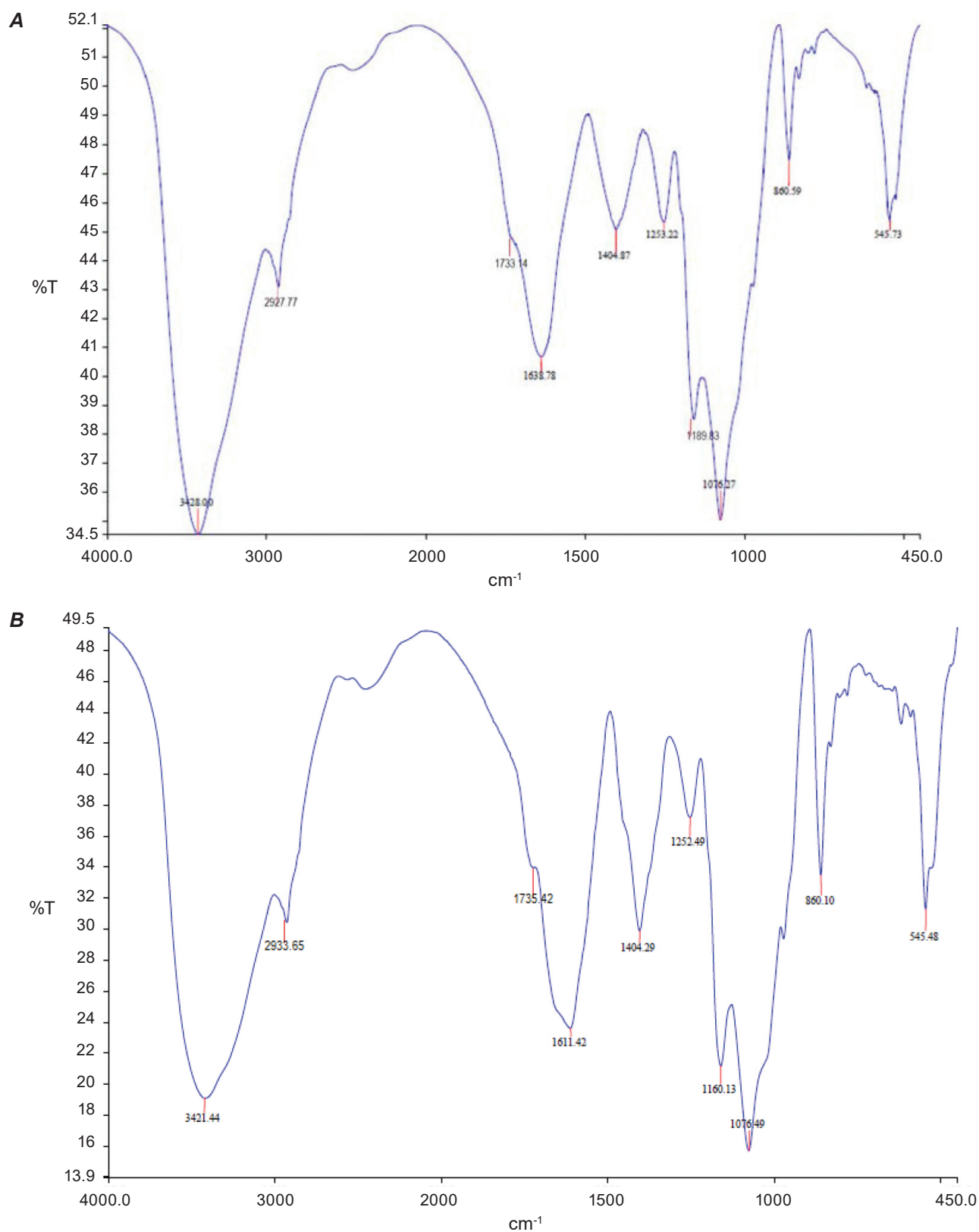


Fig. 6. FT-IR spectrum of xanthan samples (A) standard xanthan powder (Sigma-Aldrich®), and (B) Xanthan product of Xci/NIGEB-386



ver, to achieve the expression level of each of these genes, techniques such as Real-time PCR should be performed [23].

Remarkable apparent viscosity was observed in the culture broth of both strains at the end of fermentation. The purified xanthan viscosity of *Xci*/NIGEB-386 was more than xanthan viscosity produced by *Xcc*/DSM1706; however, it was approximately equal to one second of the commercial xanthan (Sigma-Aldrich) (Table 3). Research has shown that increasing the acetate and pyruvate contents in the xanthan structure have very significant effects on increasing its viscosity [21]. However, our results showed that acetate content is relatively similar in both xanthan samples. Although the type of microorganism and the environmental conditions are two important factors affecting the structure of xanthan, the rheological properties of xanthan caused by acetate in increasing the viscosity are consistent with the results of Rodriguez et al., [25]. Other studies have also shown that high content of pyruvate and lower acetate increases the viscosity of the xanthan solution [28]. Therefore, with increasing pyruvate content (to 0.1-0.5%), viscosity also increases by about 60 to 80%.

The present study showed that there is a direct correlation between pyruvate content and viscosity, because pyruvate content and viscosity of 1% (w/v) solution of standard xanthan is about 2.5 times higher than the pyruvate content and viscosity of xanthan obtained from strain NIGEB-386. The pyruvic acid content appears to be the best indicator of xanthan quality, and the percentage of acetylation and pyruvation of mannose in the xanthan structure can affect viscosity and rheological properties [4]. Although the environmental condition is an important factor in structural variation and rheological properties of xanthan [29], it does not affect the initial structure of xanthan but instead changes the formation of the xanthan's secondary structure, molecular weight, and production efficiency [5, 6].

FTIR is a method for detecting similarities and differences in the chemical structure of compounds [9, 22, 30]. In spite of minor differences of obtained peaks in the FTIR spectra, the results of this study showed that the infrared spectrum of xanthan produced by strain NIGEB-386 is very similar to that of the purified xanthan (Sigma-Aldrich®). Silva et al., [28] evaluated two strain *X. campestris* 1866 and 1867 for xanthan production and showed that using different carbon sources and strains, the FTIR spec-

tra in each of the two strains have similar functional groups.

These results confirmed that compared to the commercial strain *X. campestris*, the use of strain *Xci*/NIGEB-386 for producing xanthan in lactose-enriched medium such as cheese whey, could be promising. As further studies, the optimization of factors affecting growth and xanthan production, such as nitrogen, mineral resources, aeration rate and initial inoculation to improve the fermentation process, are continued.

*Conflict of interest.* Authors have completed the Unified Conflicts of Interest form at [http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi\\_disclosure.pdf](http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf) and declare no conflict of interest.

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### ФІЗИКО-ХІМІЧНІ ХАРАКТЕРИСТИКИ І ОДЕРЖАННЯ КСАНТАНОВОЇ КАМЕДИ ЗА ДОПОМОГОЮ НАТИВНИХ ІЗОЛЯТІВ *Xanthomonas citri* subsp. *citri*, ЯКІ ПЕРЕТВОРЮЮТЬ ЛАКТОЗУ

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Ксантан – це біополімер, що виробляється бактеріями *Xanthomonas* і який широко використовується в багатьох галузях промисловості (в харчовій та маслоробній). У роботі вивчали три штами *Xanthomonas* (*X. citri*/NIGEB-88, *X. citri*/NIGEB-386 і *X. citri*/NIGEB-K37) на предмет оцінки їх промислового потенціалу за отримання ксантанової камеди в молочно-сироватковому середовищі. В процесі ферментації вимірювали швидкість росту бактерій, в'язкість, кількість біомаси, суху масу одержаного ксан-

тану і активність  $\beta$ -галактозидази. Присутність генів  $\beta$ -галактозидази оцінювали методом ПЛР. Штам NIGEB-386 виявив найкращу здатність перетворювати лактозу в молочно-сироватковому середовищі. Найбільші значення кількості і в'язкості ксантану становили 22,7 г/л і 2066,6 мПа·с відповідно. Було підтверджено наявність шести генів  $\beta$ -галактозидази в штаммах NIGEB-386 і NIGEB-K37. Вміст пірувату і ацетату в ксантановій камеді становив 2,1 і 0,29% відповідно. За допомогою інфрачервоної Фур'є-спектроскопії було визначено положення функціональних груп у структурі продукту ферментації. У молочно-сироватковому середовищі показники двох штамів NIGEB-386 і NIGEB-K37 були кращими порівняно з *X. campestris*. Показано, що *Xanthomonas citri*/NIGEB-386 придатний для промислового виробництва ксантану з використанням молочної сироватки як недорогого середовища.

**Ключові слова:** *Xanthomonas citri*, ксантанова камедь, молочна сироватка,  $\beta$ -галактозидаза, вміст пірувату і ацетату, інфрачервона Фур'є-спектроскопія.

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