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Original article

EFFECT OF SOME PHYSIOLOGICAL FACTORS ON XANTHAN PRODUCTION BY LOCALLY ISOLATED AND IDENTIFIED *Xanthomonas campestris* USING DATE EXTRACT AS A CARBON SOURCE

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ABSTRACT

Xanthan gum is a microbial polysaccharide that is used in food, medicinal, and industrial applications due to its rheological properties. Using agricultural waste, such as date extract, as a carbon source is a cost-effective way of increase xanthan production. The purpose of this study was to isolate and identify *Xanthomonas campestris* from different cruciferous plants, as it causes black rot disease. Out of the 100 original bacterial isolates, 19 were selected for PCR analysis after undergoing biochemical and Gram staining. Using gene-specific amplification, only two isolates were identified as *Xanthomonas campestris*. Cultivation on potato sucrose peptone agar (PSPA), these verified strains displayed mucoid, convex, and yellow colonies that were suggestive of xanthan gum production. The two strains of bacteria were used to produce xanthan gum and to analyze how changes of sugar concentration, incubation period, and nitrogen source affect the bacterium's ability to produce xanthan. At the end of each experiment, the residual sugar, final pH, xanthan amount, and Biomass were measured. The highest amount of xanthan gum was yielded in 5% date extract medium after 5 days of incubation using 0.12 % di-ammonium hydrogen phosphate as a nitrogen source, and yeast extract 0.3 %. The yields were 0.54 g/100 ml for strain 1 and 1.02 g/100 ml for strain 2 at a shaking incubator agitation rate of 150 rpm of the shaking incubator. This study highlights the importance of controlling physiological parameters to maximize xanthan production from local microbial resources and of synthesizing xanthan on an industrial scale from date extract.

KEYWORDS: *Xanthomonas campestris*; Xanthan gum; Date extract; Factors; Fermentation; PCR.

1. INTRODUCTION

Microbial polysaccharides have different industrial applications. They are widely used in the food, chemical, petroleum, and pharmaceutical industries. Specific microbial polysaccharides have shown remarkable medicinal value as potent immunomodulatory, anti-inflammatory, antibacterial, and even anticancer medications in addition to their dripping and thickening properties (Kiddane, 2021). Commercial strains of *X. campestris*, which belong to the aerobic Gram-negative pathogenic bacterial family, produce the exopolysaccharide "xanthan gum" (Hassanisaadi *et al*., 2024). Bacterial polysaccharides, like xanthan, or E415 as it is more widely known, have the molecular formula C35H49O29 and are composed of a repeating unit of pentasaccharides made of glucose, mannose, and glucuronic acid in a 2:2:1 ratio (Zahović *et al.*, 2024). The structure of xanthan also contains residues of pyruvate and acetate. The different levels of acetate and pyruvate are significantly influenced by both upstream and downstream production processes (Moravej *et al*., 2024). Adequate emulsion stability, solubility in both basic and acidic liquids, resistance to pH changes, and endurance to high temperatures make xanthan gum an essential biopolymer. Xanthan gum is utilized in the oil recovery, paper, textile, pharmaceutical, culinary, and cosmetic industries due to its high pseudo-plasticity and viscosity, even at low concentrations (Furtado, 2022).

It is expected that xanthan gum production will reach 80,000 metric tons worldwide, growing at a rate of 5-10% annually. (Gunasekar *et al*., 2014). Important parameters in the production of xanthan gum include the bacterial strain and the physicochemical properties of the fermentation media, such as temperature, pH, agitation, and the addition of growth stimulants (Bhat *et al.*, 2022). The cost of producing xanthan gum has recently increased due to the high cost of carbon sources, such as glucose and sucrose, used by the bacterium X. *campestris* (Li *et al*., 2016). Thus, the synthesis of xanthan gum necessitates the discovery of more cost-effective carbon sources, such as barley, sugarcane, beet molasses, date syrup, maize flour, hydrolyzed rice agro-industrial waste, and whey (Kaur *et al*., 2024). Black rot is a severe disease caused by *X. campestris*, which infects crucifers such as Brassica and Arabidopsis (Gupta et al., 2013). After invading the xylem, *X. campestris* colonizes the mesophyll. Resulting in necrosis, marginal leaf chlorosis, and darkening of the leaf veins and vascular tissue of the stem. As the disease progresses, wilting, yellowing, and complete leaf necrosis may appear (Mduma *et al*., 2015). Significant amounts of rapidly fermentable sugar, such as sucrose or glucose, are present in the medium that supports the growth of *X. campestris* to produce

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xanthan. The fermentation process is typically conducted aerobically at 28 to 30 °C with an aeration rate exceeding 0.3 v/v min-1 (Miranda *et al*., 2020). Usually, potassium, iron, magnesium, and calcium salts are added to the fermentation medium along with the carbon source. According to Kumar *et al*. (2024), xanthan gum is nontoxic, biocompatible, biodegradable, and bio-adhesive. Their use in food, pharmaceutical, biological, and cosmetic applications can be attributed to these characteristics (Singhvi *et al*., 2019). Over the past 20 years, xanthan gum has attracted much attention since the US Food and Drug Administration (FDA) authorized it as a safe food component in 1969 (Rakshit *et al*., 2024).

This study was designed to investigate the viability of using the inexpensive date extract molasses as a basal medium and carbon source for the manufacture of "xanthan gum" by a strain of the bacteria *X. campestris.* The study contributes to the development of a cost-effective and sustainable bioprocess. Additionally, using a locally isolated and identified strain could boost waste valorization efforts and regional industries while increasing production efficiency. The central research question is: When date extract is used as the primary carbon source during cultivation, what do specific physiological factors like incubation period, nitrogen supplementation, carbon source concentration, and yeast extract do to the production of xanthan gum by locally isolated and identified *X. campestris*.

2. MATERIALS AND METHODS

Isolation of bacteria:

A total of 100 samples of infected cruciferous plants from different locations in Duhok city were collected, including Duhok, Sumel, Seje, Akre, and Amedi. The samples were selected based on the symptoms of the disease, including V-shaped lesions, blackening of the veins, and drying lesions. Samples were collected from different cruciferous plants, including arugula, turnip, cauliflower, radish, cabbage, and broccoli. The samples were collected in sterilized tubes and brought to the laboratory for culturing after 2-5 hours of collection. They were sterilized using 70% ethanol for 20 seconds and washed with 3 changes of distilled water for 5 minutes each. Subsequently, the leaf samples were cut into small pieces using a sharp blade and put in phosphate-buffered saline (PBS) for 30 minutes to get a bacterial suspension. Next, bacterial suspension was cultured on potato sucrose peptone agar (PSPA) medium, which consists of (g/l) potato, 300; sucrose, 20; NaNO3, 0.2; KH2PO4, 0.3; peptone, 2; Ca (NO3)2, 0.5; KCl, 0.05; FeSO4, 0.05; agar, 20; and distilled water, 1 litter (Akhtar, 1989), using streaking methods and incubated at 28°C for 5-6 days.

Gram Staining:

The Gram stain procedure was performed to differentiate between Gram-positive and Gram-negative bacteria.

Biochemical identification:

Since PSPA medium is semi-selective, i.e., not selective for X. campestris, different types of bacterial colonies were grown on it. Based on different microbiological tests, the targeted bacterium was selected. Biochemical tests, such as oxidase and catalase, were performed on each Gram-negative colony grown on PSPA medium to select suspected colonies for further PCR identification.

Molecular identification:

DNA extraction was performed using the boiling method: colonies from the suspected bacterial samples were picked up, placed in an Eppendorf tube containing 200 µl of sterile water, and vortexed thoroughly. The tubes were labelled and put on a hot plate at 95°C for 20 minutes. Subsequently, the samples were cooled immediately by transferring them to a refrigerator for 5 minutes. After that, the tubes were centrifuged at 15000 rpm for 2 minutes, and the supernatant, which contained DNA, was collected (Chen & Kuo, 1993). The concentration of suspected DNA samples was measured using a DeNovix (Wilmington, USA) NanoDrop spectrophotometer. The purity ratio of 260/280 was falling between 1.6 and 2.4. According to previous studies, the majority of research has focused on the *hrpF* gene for the identification of *X. campestris.* The specific primers: XCF(5′-CGATTCGGCCATGAATGACT-3′) and XCR (5′-CTGTTGATGGTGGTCTGC AA-3′) were designed from *the hrpF* gene of *X. campestris*, with a predicted PCR product of 535 bp (Park *et al*., 2004). PCR assays were performed with a GeneAmp PCR System 9700 Thermocycler (Thermo Fisher Scientific, USA). All amplifications were carried out in a final volume of 20 µl containing 10 µl of master mix (which contains dNTPs, $MgCl_2$, BSA buffer, and Taq polymerase),1.5 µl of forward primers, 1.5 µl of reverse primers, 1.5 µl of DNA samples, and 5.5 µl of distilled water. Reactions were run for 35 cycles, each consisting of 30 seconds at 94°C, 30 seconds at 58°C, and 30 seconds at 72°C, with an initial denaturation of 5 minutes at 94°C and a final extension of 5 minutes at 72 °C. An 8-ml aliquot of each amplified PCR product was electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV transillumination (Park *et al*., 2004).

Inoculum preparation:

A three-day-old bacterial culture was used to inoculate 250-ml conical flasks containing 100 ml of PSPA broth to create the inoculum. The inoculated flasks were incubated at 28 ± 1°C and 150 rpm in a shaking incubator for 72 hours. Each prepared fermentation medium used for xanthan synthesis was inoculated with this inoculum.

Growth medium:

The growth medium was prepared using date extract syrup as a carbon source. The sugar concentration in date syrup was 65%, and different concentrations were prepared from it. 0.15% $NaNO_3$ was added as a nitrogen source. The broth medium was well mixed, and the pH was adjusted to 7.0.

Cultural conditions:

The growth medium for each experiment was prepared and dispensed into 250 ml Erlenmeyer flasks in triplicate, each containing 45 ml of broth medium. Afterwards, they were plugged and autoclaved at 121°C for 15 minutes. Followed by adding 5% of the bacterial cell suspension to the culture flasks once they had cooled. The inoculation culture flasks were then incubated at 28 ± 1 ºC in a shaking incubator at 150 rev/min.

Analytical methods:

Triplicate samples were collected at regular intervals throughout fermentation to assess growth, xanthan production, residual sugar levels, and final pH.

Determination of Xanthan Concentration:

The amount of xanthan gum was estimated by centrifuging the fermentation medium (ROTOFIX 32A, Germany) at 5000 rpm for 20 minutes. The supernatant containing xanthan was collected. Xanthan gum was precipitated from the supernatant by adding two volumes of cold ethanol. The mixture was then dried at 50°C until a constant weight was recorded (Hu *et al*., 2019).

Determination of Initial and Residual Sugar:

Using glucose as a standard, the phenol-sulfuric acid method or the Dubois method (DuBois *et al.,* 1956) was used to determine initial and residual sugar concentrations. 1 mL of sample was placed in a glass tube and mixed with 1 mL of 5% phenol. Then, 2.5 mL of sulfuric acid was added. Afterward, the tube was put in a shaking water bath at 25°C for 30 min. Then, the absorbance was measured at 490nm using a spectrophotometer (JENWAY 630, UK).

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3. RESULTS

Biomass determination:

The biomass of the bacterial isolate was extracted from the fermentation medium via centrifugation at 10,000 rpm for 10 minutes. The generated biomass was then washed with sterile distilled water and left suspended for 2 hours. It was then precipitated for 20 minutes at 6000 rpm. The precipitated cells were then dried for 24 hours at 65°C in an oven. A sensitive balance (KERNPFB, Germany) was used to determine the dry weight of the biomass.

Statistical analysis:

All data are presented as mean ± SD (Tables 1, 2, and 3). GraphPad 5.05 was used to investigate the statistical correlation between xanthan production of xanthan and cultural conditions (incubation period, nitrogen sources, and different sugar concentrations); $P < 0.05$.

Identification of bacteria:

After initial screening for potential *X. campestris* isolates using Gram staining and biochemical assays, 19 samples out of 100 were chosen because they exhibited physiological and morphological characteristics in common with X. campestris, including Gram-negative rod-shaped cells, negative oxidase, and positive catalase activity. PCR amplification was carried out using specific primers to *X. campestris.* The results in the figure. 1 indicates that only two of the 19 strains tested positive for *X. campestris*-specific bands, confirming their molecular identity**.** After molecular verification, Biochemical tests were performed for these two strains. Two bacterial samples were obtained from different vegetable sources. Sample A (Sample No. 60) was extracted from cauliflower that was collected in Seje/Duhok, whereas Sample B (Sample No. 79) was isolated from turnip in Masik/Duhok. The catalase enzyme, which breaks down hydrogen peroxide into oxygen and water, was present in both isolates, as evidenced by their positive catalase test results and negative oxidase test findings. Cytochrome c oxidase activity was also absent.

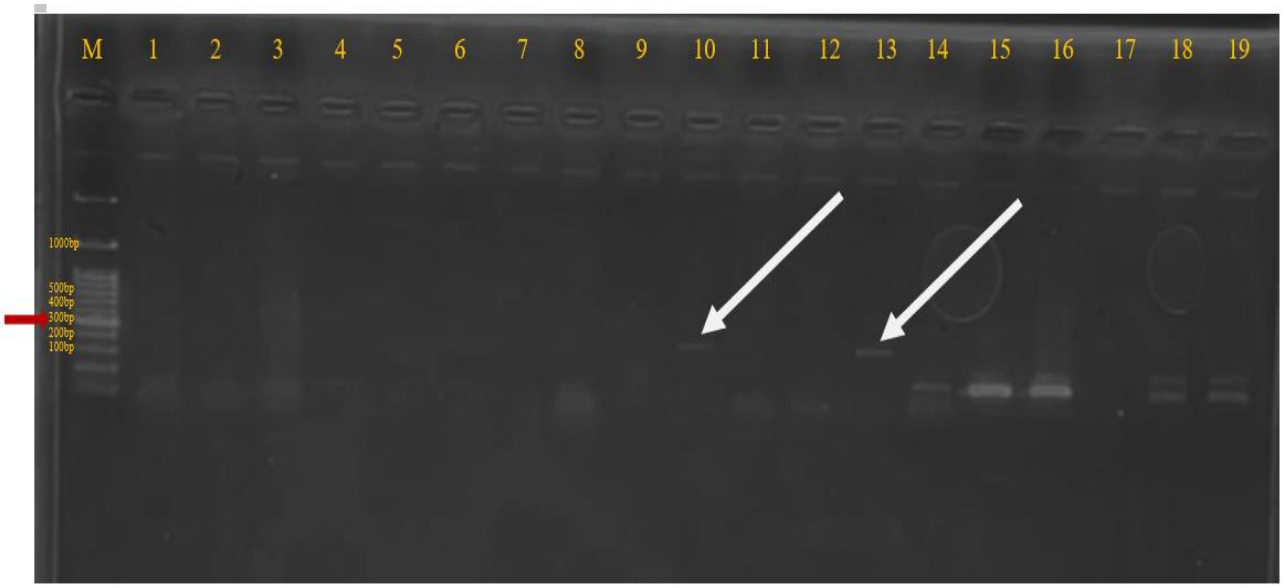

Figure 1: Conventional PCR for identifying *the hrpF* gene of *X. campestris*. M: marker (100 bp DNA ladder); lanes 1 to 19 are suspected samples. Clear bands of approximately 535 bp were observed, which correspond to the expected amplified *hrpF* gene.

The two strains were subcultured on PSPA medium. *X. campestris* strains 1 and 2 showed similar morphological characteristics on the culture medium. In strain 1, the round colonies had entire margins, but in strain 2, the edges were entire to somewhat uneven. The formation of xanthomonadins, the characteristic yellow pigments of *X. campestris*, is compatible with the rod-shaped cells of both strains and the colour of the colonies, which ranged from yellow to creamy. Colonies were raised to convex for both strains. Gram staining confirmed that both isolates were Gram-negative, as is typical for this species.

The effect of different incubation periods on xanthan production:

In this experiment, the bacterium was tested to determine the most suitable day for the highest xanthan gum production. The experiment was performed over 6 days, starting from day 3. The growth, xanthan production, sugar uptake, and final pH of X. campestris in the fermentation medium were measured, as shown in Table 1. Strain 1 of this bacterium produced 0.28 g/100 ml of xanthan after two days of incubation. The xanthan gum concentration peaked at 0.40 g/100 mL on day 5 and remained at that level for the duration of the trial. Production fell to 0.35 g/100 ml after six days. For strain 2, xanthan production increased incrementally, peaking at 0.98 g/100 mL after 5 days. After 6 days, the output decreased to 0.84 g/100 mL A comparison between strains reveals that strain 2 produces more xanthan than strain 1, which may be attributed to its greater enzymatic activity in the xanthan biosynthetic pathway.

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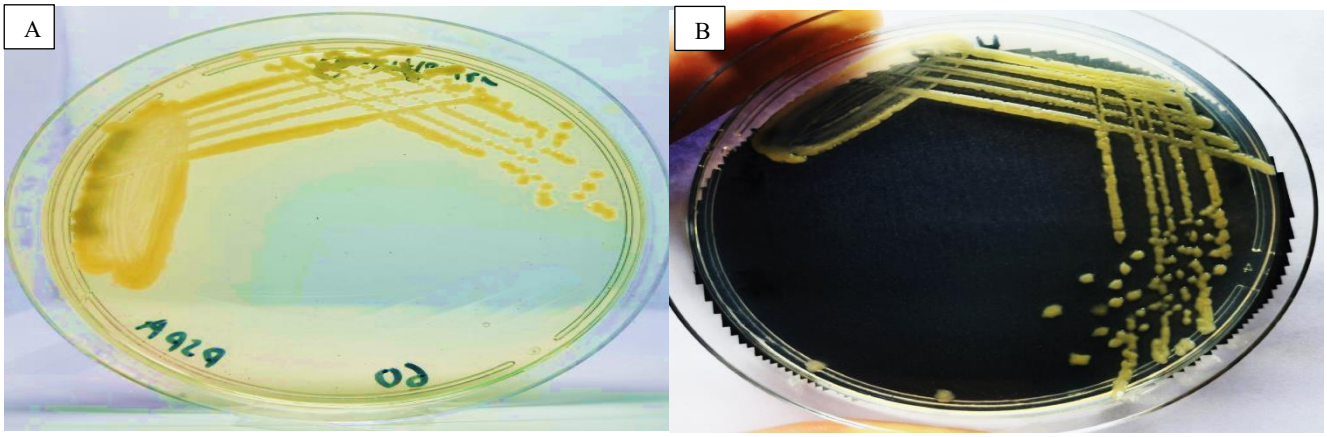

Figure 2: Identified strains on PSPA medium show small, yellow, mucoid colonies due to xanthan production.

Table 1: The effect of different incubation periods on xanthan production by *X. campestris*

Bacterial strain	Incubation day	Biomass g/100ml	Xanthan g/100 ml	Residual sugar %	Final pH
strain 1	3	0.38 ±0.011	0.28 ±0.011	3.44 ±0.050	5.1 ±0.057
	4	0.42 ±0.015	0.34 ±0.005	2.61 ±0.076	5.5 ±0.100
	5	0.50 ±0.020	0.40 ±0.020	1.45 ±0.050	5.6 ±0.152
	6	0.48 ±0.015	0.35 ±0.049	1.04 ±0.058	5.9 ±0.057
strain 2	3	0.39 ±0.020	0.33 ±0.010	3.61 ±0.079	5.3 ±0.152
	4	0.48 ±0.010	0.42 ±0.015	2.47 ±0.030	5.4 ±0.100
	5	0.97 ±0.005	0.98 ±0.010	1.37 ±0.025	5.5 ±0.100
	6	0.95 ±0.010	0.84 ±0.026	1.17 ±0.061	6.0±0.057

Each number represents the mean of three replicates and standard deviation ± SD

The Effect of Different Sugar Concentrations on Xanthan Production:

Different strains of *X. campestris* produce xanthan gum with varying sugar concentrations. Different sugar concentrations (1%, 3%, 5%, 7%, and 9%) were used. The results are listed in Table 2. Both strains showed a steady increase in xanthan yield as the sugar content increased from 1% to 5%. From 0.14 g/100 ml (1%) to 0.38 g/100 ml (5%) by strain 1. From 0.20 g/100 ml (1%) to 0.98 g/100 ml (5%) by strain 2. Increased carbon availability improves polysaccharide synthesis. The highest xanthan production was recorded for strain 1 (0.38 g/100 ml) and strain 2 (0.98 g/100 ml) at 5% sugar concentration. Strain 1: The residual sugar ranges from 0.20% (1%) to 3.08% (9%). At every stage, strain 2 showed slightly less residual sugar than strain 1. Furthermore, biomass increased in tandem with sugar concentration: strain 1, 0.18–0.64 g/100 mL; strain 2, 0.18–1.08 g/100 mL.

Table 2: The effect of different sugar concentrations on xanthan production by *X. campestris.*

Bacterial strain	Sugar concentration%	Biomass g/100 ml	Xanthan g/100 ml	Residual sugar %	Final pH
strain 1	1	0.18 ±0.015	0.14 ±0.020	0.20 ±0.005	5.6 ±0.152
	3	0.40 ±0.005	0.34 ±0.010	1.17 ±0.055	5.3 ±0.057
	5	0.47 ±0.010	0.38 ±0.005	1.32 ±0.036	5.4 ±0.057
	7	0.56 ±0.015	0.31 ±0.010	2.44 ±0.036	5.3 ±0.057
	9	0.64 ±0.010	0.28 ±0.010	3.08 ±0.060	5.3 ±0.057
strain 2	1	0.18 ±0.010	0.20 ±0.015	0.16 ±0.015	5.5 ±0.057
	3	0.54 ±0.010	0.45 ±0.015	1.02 ±0.025	5.4 ±0.100
	5	0.99 ±0.010	0.98 ±0.010	1.41 ±0.095	5.4 ±0.057
	7	0.97 ±0.015	0.65 ±0.030	2.55 ±0.015	5.4 ±0.057
	9	1.08 ±0.010	0.56 ±0.015	3.27 ±0.055	5.4 ±0.057

Each number represents the mean of three replicates and standard deviation ±SD.

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The Effect of Different Nitrogen Sources on Xanthan Production:

For both strains, $(NH_4)_2HPO_4$ was the most effective nitrogen source for xanthan production. Across all nitrogen sources, strain 2 was more productive than strain 1. Nitrogen supply affects the final pH and sugar consumption efficiency, two factors crucial for optimizing fermentation conditions, as well as xanthan yield. The results are in Table 3. Using $NH_4)_2HPO_4$, the amount of xanthan produced reached 0.53 g/100 ml by strain 1 and 1.01 g/100 ml by strain 2, followed by sodium nitrate, with xanthan yield at 0.38 g/100 ml by strain 1 and 0.99 g/100 ml by strain 2. Urea increased the xanthan yield to 0.31 g/100 mL by strain 1 and 0.78 g/100 mL by strain 2.

Table 3: The effect of different nitrogen sources on xanthan production by *X. campestris.*

Bacterial strain	Nitrogen source %	Biomass g/100 ml	Xanthan g/100 ml	Residual sugar %	Final pH
strain 1	$NaNO_3$ (0.15)	0.47 ±0.010	0.38 ±0.005	1.32 ±0.036	5.4 ±0.057
	Peptone (0.15)	0.46 ±0.015	0.32 ±0.208	1.21 ±0.100	5.9 ±0.057
	$(NH_4)_2HPO_4$ (0.12)	0.50 ±0.010	0.53 ±0.005	0.99 ±0.100	5.8 ±0.057
	NH_4CL (0.10)	0.42 ±0.015	0.30 ±0.010	1.14 ±0.015	5.1 ±0.057
	Urea (0.07)	0.49 ±0.015	0.31 ±0.005	1.10 ±0.015	5.3 ±0.057
strain 2	$NaNO_3$ (0.15)	0.97 ±0.010	0.99 ±0.010	1.43 ±0.102	5.4 ±0.100
	Peptone (0.15)	0.56 ±0.015	0.72 ±0.0100	1.21 ±0.0100	5.9 ±0.100
	$(NH_4)_2HPO_4$ (0.12)	0.97 ±0.010	1.01 ±0.0100	0.93 ±0.020	5.8 ±0.100
	NH_4CL (0.10)	0.48 ±0.010	0.41 ±0.020	1.14 ±0.015	5.1 ±0.057
	Urea (0.07)	0.92 ±0.025	0.78 ±0.015	1.05 ±0.025	5.4 ±0.057

Each number represents the mean of three replicates and standard deviation ±SD

The Effect of Different Concentrations of Yeast Extract on Xanthan Production:

Yeast extract, which stimulates xanthan production, was used in this experiment with different concentrations: (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%). The results are presented in Table 4. A possible shift in metabolic priority from xanthan production to cell growth was indicated by greater amounts of yeast extract, even though biomass growth was not necessarily correlated with higher xanthan yield. The final pH and residual sugar measurements indicate that efficient sugar utilization was achieved at the optimal yeast extract concentration. The biomass of the two strains increased with increasing yeast extract concentration. The biomass of strain 1 increased from 0.44 to 0.66 g/100 ml, while that of strain 2 increased from 0.55 to 0.98 g/100 ml, achieving this more rapidly and peaking at 0.3%. If the extract levels exceed 0.3%, bacterial growth will be inhibited. The production of xanthan gum by strain 1 peaked at 0.54 g/100 ml at 0.3% yeast extract and declined at higher concentrations (0.4–0.5%).

Table 4: The effect of different concentrations of yeast extract on xanthan production by *X. campestris.*

Bacterial strain	yeast extract%	Biomass g/100 ml	Xanthan g/100 ml	Residual sugar %	Final pH
strain 1	0.1	0.44 ±0.005	0.29 ±0.100	3.11 ±0.100	5.3 ±0.100
	0.2	0.52 ±0.025	0.32 ±0.100	2.93 ±0.080	5.3 ±0.100
	0.3	0.65 ±0.005	0.54 ±0.005	2.99 ±0.010	5.8 ±0.152
	0.4	0.62 ±0.100	0.31 ±0.010	2.41 ±0.147	5.1 ±0.057
	0.5	0.66 ±0.010	0.30 ±0.005	1.59 ±0.015	5.5 ±0.100
strain 2	0.1	0.55 ±0.020	0.32 ±0.010	3.06 ±0.066	5.2 ±0.057
	0.2	0.75 ±0.020	0.50 ±0.005	2.85 ±0.060	5.0 ±0.057
	0.3	0.98 ±0.010	1.02 ±0.010	3.06 ±0.058	5.3 ±0.152
	0.4	0.86 ±0.015	0.54 ±0.010	2.36 ±0.080	5.1 ±0.057
	0.5	0.88 ±0.010	0.51 ±0.010	1.56 ±0.010	5.5 ±0.057

Each number represents the mean of 3 replicates and standard deviation ±SD.

4. DISCUSSION

The effects of particular physiological parameters, such as the amounts of carbon, nitrogen, and yeast extract, on the formation of xanthan gum by locally isolated and identified strains of *X. campestris* were evaluated using date extract as a carbon source. Date extract, a natural and affordable substrate, provided an alternative to commercial sugars. The amount of xanthan produced was strongly influenced by the type and concentration of nutrients provided. Regarding the incubation period, a Comparison between strains shows that strain 2 produces more xanthan than strain 1, which may be associated with greater enzymatic activity in the xanthan biosynthetic pathway. Results

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showed that there is a dependency between the growth of the bacteria and their exopolysaccharide production, growth, and sugar consumption. These results are consistent with those of Souw and DemaiN (1979), who reported a maximum in xanthan synthesis after 5 days of incubation. Additionally, Makut *et al.* (2024) reported a maximum xanthan production of 1.38 g/L after 5 days of incubation. Sidkey *et al.* (2020) also reported that the highest xanthan production, achieved after 5 days under optimized conditions, was 16.35 g/L. Accumulating toxic lipids may affect the metabolic pathway of xanthan formation, which could explain the decline in xanthan production. Furthermore, the use of some sugar for the production of energy essential to bacterial growth and anabolic processes may explain the low rate of xanthan accumulation at the start of incubation periods. Increasing bacterial cell count encouraged the synthesis of more xanthan.

The amount of sugar in the culture medium affected the bacterium *X. campestris'* ability to produce xanthan. The biosynthesis of xanthan was induced by gradually increasing the concentration of added sugar in the culture medium. However, across all concentrations, strain 2 consistently produced more xanthan, indicating a greater biosynthetic capacity. Above 5% sugar concentration, xanthan production begins to decrease due to factors such as high osmotic pressure, catabolite repression, increased viscosity of the medium, and accumulation of toxic metabolites. The remaining sugar increases as the input sugar does, but not proportionally, indicating that a significant amount of sugar is digested. The increased efficiency of sugar uptake or utilization in strain 2 may be the cause of its superior xanthan synthesis. More carbon promotes cell growth; however, strain 2 devotes a larger percentage of this metabolic activity to xanthan production than to biomass. The production of xanthan gum is inhibited when the sugar content of the culture medium exceeds 5%. According to Lo *et al*. (1997), *X. campestris* produced the most xanthan when the culture medium contained 5% sugar in the form of glucose. Ozdal and Başaran (2019) utilized 4% sugar beet molasses as a carbon source, achieving a maximum xanthan production of 20.5 g/L. (Li *et al*., 2012) used 3% cassava starch as a carbon source, which strongly stimulated xanthan production. De Sousa Costa *et al*. (2014) found that using 10% shrimp shells produced the highest xanthan gum concentration (4.64 g/L). *X. campestris* enhanced the production of xanthan by applying most nitrogen sources to the date extract medium. The most superior one is 0.12% di-ammonium hydrogen phosphate; these findings are consistent with those reported by Cadmus et al. (1978), who stated that 0.15% di-ammonium hydrogen phosphate stimulates higher xanthan production. Compared with the outcomes of experiment 3.3, the fermentation medium containing NH4CL is the only nitrogen source that inhibits xanthan synthesis. Because xanthan gum is acidic and contains organic acids, it typically accumulates in the culture medium, which explains why the pH of the fermentation medium pH decreases. Urea is also a good nitrogen source for xanthan production when added to the fermentation medium. Kassim (2011) also employed urea at a 4% concentration to increase the bacterial strain's synthesis of xanthan. According to Carignatto *et al*., (2011), higher xanthan was produced after the addition of 0.25% di-ammonium hydrogen phosphate to the fermentation medium. Rashidi *et al*. (2023) stated that the maximum xanthan production was achieved using 1.2% di-ammonium hydrogen phosphate, which resulted in 12.5 g/L.

Both strains produced the highest amount of xanthan at 0.3% yeast extract. Growth and xanthan production are both significantly impacted by yeast extract; for both strains, a concentration of 0.3% is ideal. These results are consistent with those of Kongruang (2013), who used a range of concentrations of yeast extract concentrations (0.1% to 0.5%) and found that the maximum amount of xanthan production occurred at 0.3%. According to comparative studies, the addition of yeast extract to the production medium effectively increased the yield of xanthan (Gomashe, 2013). These findings highlight the importance of medium optimization in enhancing microbial polysaccharide synthesis in low-cost systems.

CONCLUSION

Date extract can be used as a carbon source for xanthan gum production, serving as an excellent, cost-effective substrate that replaces synthetic media. Based on this study, incubation for 5 days yielded the highest xanthan production. The combination of 5% sugar concentration and $(NH_4)_2HPO_4$ as a nitrogen source and 0.3% yeast extract favoured the production of the highest yield of xanthan. This study demonstrates how xanthan gum production can be enhanced from both financial and environmental sustainability standpoints by utilizing readily accessible raw materials. Strain-dependent differences in xanthan production highlight the importance of selecting the appropriate strain in biopolymer fermentation processes. Overall, this work supports the use of agricultural by-products in biotechnological production and encourages further optimization for xanthan synthesis on a commercial scale.

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According to institutional and publication rules, no ethical clearance or approval was required because the research did not involve human subjects or animal models.

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Conflicts of interest:

The authors stated that there is no potential conflict of interest in this study.

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