



افزایش تولید صمغ زانتان با استفاده از ملاس به عنوان منبع کربن در فرآیند تخمیر توسط *Xanthomonas campestris*

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چکیده

صمغ زانتان، یک پلی ساکارید میکروبی است که توسط *Xanthomonas campestris* تولید می شود و به دلیل خواص رئولوژیکی منحصر به فرد خود به طور گسترده ای در صنایع مختلف استفاده می شود. انتخاب محیط رشد نقش حیاتی در به حداکثر رساندن تولید زانتان دارد. این مطالعه با هدف بررسی اثر محیط های کشت مختلف بر تولید زانتان و همچنین ارزیابی ملاس به عنوان یک محیط کشت مقرون به صرفه و کارآمد برای سویه های *Xanthomonas campestris* انجام شده است. در این مطالعه، تولید زانتان توسط دو سویه *X. campestris* با استفاده از دو محیط مختلف، محیط NB و ملاس، مورد ارزیابی قرار گرفت. صمغ زانتان با استفاده از ایزوپروپانول و کلسیم کلرید رسوب داده شد و سپس با سانتریفیوژ برای اندازه گیری بازده جداسازی شد. نتایج نشان داد که تفاوت های قابل توجهی در تولید زانتان بین دو محیط وجود دارد. سویه ۱ در محیط NB مقدار ۸۰۰ گرم در لیتر زانتان تولید کرد، در حالی که همین سویه در محیط حاوی ملاس ۴۵/۴۰ گرم در لیتر زانتان تولید نمود. هم چنین، سویه ۲ نیز در محیط NB مقدار ۹/۶۰ گرم در لیتر تولید کرد، در حالی که در محیط حاوی ملاس بالاترین بازده با ۵۲/۰۰ گرم در لیتر مشاهده شد. این یافته ها نشان می دهد که ملاس به دلیل محتوای بالای کربن، تولید زانتان را در مقایسه با محیط NB به طور قابل توجهی افزایش می دهد. این مطالعه پتانسیل استفاده از ملاس را به عنوان یک بستر مقرون به صرفه برای تولید صنعتی زانتان، به ویژه با استفاده از سویه های با بازده بالا، تایید می کند. بهینه سازی بیشتر پارامترهای تخمیر می تواند به بازدهی تولید بالاتری نیز منجر شود.

واژگان کلیدی: تولید صمغ زانتان، *Xanthomonas campestris*، تخمیر ملاس، بهینه سازی محیط رشد.

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Enhanced Xanthan Gum Production Using Molasses as a Carbon Source in *Xanthomonas campestris* Fermentation

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Abstract

Xanthan gum, a microbial polysaccharide produced by *Xanthomonas campestris*, is widely used in various industries due to its unique rheological properties. The choice of growth medium plays a crucial role in maximizing xanthan production. The aim of this study is to assess the effect of different growth media on xanthan production and to explore molasses as a cost-effective and efficient substrate for *Xanthomonas campestris* strains. In this study, we evaluated the production of xanthan by two *X. campestris* strains using two different media: Nutrient Broth and molasses. Xanthan gum was precipitated using isopropanol and calcium chloride, followed by centrifugation to measure yield. The results showed significant differences in production between the two media. Strain 1 produced 8.00 g/L xanthan in NB medium, while in molasses yielded 45.40 g/L. Similarly, strain 2 produced 9.60 g/L xanthan in NB medium, whereas this strain exhibited the highest yield of 52.00 g/L in molasses. These findings indicate that molasses, due to its rich carbon content, substantially enhances xanthan production compared to NB medium. This study underscores the potential of molasses as a cost-effective substrate for xanthan production in industrial-scale, especially with high-yielding bacterial strains. Further optimization of fermentation parameters could lead to higher production efficiencies.

Keywords: Xanthan gum production, *Xanthomonas campestris*, Molasses fermentation, Growth media optimization.

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Introduction

Xanthan gum, a high-molecular-weight polysaccharide synthesized by the bacteria such as *Xanthomonas campestris* (1). It is a

gram-negative, rod-shaped bacterium commonly found in plant environments (2). *Xanthomonas campestris* and *Xanthomonas citri* are well-known plant pathogens that cause significant agricultural damage. *Xanthomonas campestris* is primarily associated with black rot in cruciferous plants, while *Xanthomonas*

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citri causes citrus canker, a devastating disease for citrus crops. Despite their pathogenic nature, both species are extensively studied for their ability to synthesize xanthan gum (3,4). Among them, *Xanthomonas campestris* is mostly considered the primary producer of xanthan gum, valued for its efficient synthesis during aerobic fermentation (5).

Xanthan gum is extensively utilized in various industrial applications due to its distinctive rheological properties, including viscosity enhancement and stabilization of emulsions (5,6). These characteristics render xanthan gum an essential additive in the food, pharmaceutical, cosmetic, and oil recovery industries, necessitating efficient production methods to meet the increasing global demand (7). The versatility of xanthan gum has led to its widespread use in different industries. In the food industry, it is commonly employed as a thickener and stabilizer in products like salad dressings, sauces, and gluten-free baked goods (8). In agriculture, xanthan gum is employed as a carrier for herbicides and pesticides, improving their distribution and effectiveness. Its water retention properties also find use in soil conditioning and seed coatings (9,10). One of the most significant non-food applications of xanthan gum is in the oil and gas industries, where it is used as a viscosifier in drilling fluids during enhanced oil recovery (EOR) processes (11). Its property to remain stable under high temperatures and salinity makes it ideal for these environments, helping to transport oil through porous rock formations and suspend drill cuttings in oil wells (12).

The structure of xanthan gum is complex and consists of a high-molecular-weight heteropolysaccharide. Its backbone is made up of β -(1 $\rightarrow$ 4)-D-glucose units, similar to cellulose, with trisaccharide side chains

branching off every second glucose residue. These side chains are composed of two mannose units and one glucuronic acid unit (5,13). The unique properties of xanthan gum arise from the modifications in the side chains, which can be pyruvylated or acetylated (14). Pyruvyl groups are attached to the terminal mannose residues, while acetyl groups may modify the internal mannose. These chemical modifications enhance the gum's solubility, viscosity, and interaction with other compounds, such as ions in solution (8,15).

Despite its extensive use, one of the primary challenges in xanthan gum production is the high cost associated with conventional use of carbon sources such as glucose and sucrose, which make up a significant portion of production expenses (16). To address this, alternative and cost-effective substrates are being investigated. The biosynthesis of xanthan gum is highly dependent on the growth conditions of *X. campestris*, particularly the composition of the fermentation medium. Conventional nutrient broth (NB) has been widely employed for cultivating *X. campestris*, however, its limited carbon content often leads to suboptimal polysaccharide yields. This limitation highlights the need for alternative carbon sources that can enhance xanthan production and simultaneously reducing manufacturing costs as well. Among the promising candidates, molasses—a by-product of the sugar refining process—has emerged as a cost-effective and abundant substrate, rich in fermentable sugars, including sucrose, glucose, and fructose (17-19).

In this study, we aim to systematically investigate the effects of molasses as a carbon source on xanthan gum production by two distinct strains of *Xanthomonas campestris*. By conducting a comparative analysis of xanthan

yields obtained in traditional NB medium versus those in molasses-based media, this research seeks to elucidate the advantages of employing molasses in fermentation processes. By demonstrating the potential of molasses as an industrial substrate for xanthan gum production, this study underscores the critical importance of optimizing growth media to maximize production efficiency in the biopolymer industry, aligning with contemporary efforts to integrate waste materials into bioprocessing methodologies for enhanced economic viability and environmental sustainability.

Additionally, this study explores the implications of strain variability on xanthan production efficiency. Different strains of *X. campestris* can exhibit distinct metabolic capacities, influencing their effectiveness in utilizing various substrates. Understanding these strain-specific differences is essential for the selection of high-yielding strains for industrial applications and optimizing production strategies.

Materials and methods

A: Bacterial Strains and Growth Conditions: Two strains of *Xanthomonas campestris*, namely IBRC 10644 (strain1) and *Xanthomonas campestris* PV. IBRC 11092 (strain 2), were employed to assess xanthan gum production. These strains were sourced from the Iranian National Biological Resource Center (NBRC). The bacterial strains were maintained on nutrient agar (NA) plates for preservation and were stored under controlled refrigeration to ensure long-term viability. For biomass generation, the strains were cultured in Tryptic Soy Broth (TSB), incubating at 30°C for 24 hours, with shaking at 120 rpm to promote exponential growth.

B: Inoculum preparation: The inoculum was prepared by using TSB medium, which was supplemented with 20 g/L glucose, 20 g/L peptone, and 10 g/L yeast extract. The medium was autoclaved and cooled. A loopful of each bacterial strain was introduced into 50 mL of the TSB and incubated at 30°C with agitation at 120 rpm for 24 hours (20). At this stage, each strain reached the logarithmic phase (OD=1), ensuring optimal growth conditions for subsequent fermentation. The resulting inoculum was used at a concentration of 2.5% (v/v) for fermentation trials (21).

C: Xanthan gum fermentation: Xanthan gum production was carried out in two distinct media: molasses-based medium and nutrient broth (NB). The molasses medium was prepared at a concentration of 19% Brix, while the NB medium contained 2% yeast extract and 1% peptone. Both media were supplemented with the following: 2 g/L potassium dihydrogen phosphate, 0.2 g/L magnesium sulfate, 2 g/L ammonium nitrate, 2 g/L citric acid, 0.0006 g/L boric acid, 0.0006 g/L zinc chloride, 0.0024 g/L ferric chloride, and 0.02 g/L calcium carbonate. The pH of the media was adjusted to 7.2 using 1 M NaOH, and sterilization was carried out by autoclaving at 115°C for 10 minutes (20). The fermentation was conducted aerobically in 50 mL Erlenmeyer flasks with a working volume of 30 mL. The flasks were incubated at 28°C with shaking at 180 rpm for 48 hours.

D: Xanthan gum recovery: After 48 hours of fermentation, the bacterial cultures were centrifuged at 15,000 rpm for 25 minutes at 4°C to separate the cells from the xanthan-containing supernatant. The xanthan gum was precipitated by adding 1 mL of isopropanol containing 1% calcium chloride to 0.5 mL of the supernatant (22). The resulting mixture was centrifuged again at 15,000 rpm

for 30 minutes at 4°C, leading to the formation of a xanthan gum pellet.

E: Xanthan gum drying: The xanthan gum pellet was subjected to drying at 40°C for 72 hours to ensure complete removal of residual moisture. This controlled drying process minimized measurement errors and ensured the accurate determination of xanthan yield (23).

F: Xanthan yield measurement: The xanthan yield was quantified by measuring the weight of the microtubes before and after drying the xanthan gum. The difference in weight was used to calculate the amount of xanthan produced, expressed in grams per liter (g/L). To ensure precision, a balance with an accuracy of 0.0001 grams was employed for the measurements (20).

Results

The xanthan gum production data for the two *Xanthomonas campestris* strains grown in different media are summarized in Table 1. The results reveal significant variations in polysaccharide yield, highlighting the influence of the culture medium on xanthan biosynthesis. *Table 1:* In the NB medium, strain 1 produced 8 g/L of xanthan, which represents a relatively low yield. Although this medium supports bacterial growth, the suboptimal xanthan production suggests that NB may not provide the most favorable conditions for maximal polysaccharide synthesis. In contrast, when Strain 1 was cultured in molasses-based medium, xanthan yield increased substantially to 45.4 g/L. The high carbon content of molasses likely enhanced the metabolic activity of *X. campestris*, facilitating greater xanthan production. This result underscores the utility of as a cost-effective and efficient carbon source for industrial xanthan production.

Strain 2 exhibited a slightly higher xanthan yield in the NB medium, producing 9.6 g/L. This modest increase compared to Strain 1 could be attributed to genetic or metabolic differences between the two strains, potentially affecting their efficiency in utilizing available nutrients. Notably, strain 2 demonstrated the highest xanthan yield when cultured in molasses, producing 52 g/L. This finding suggests a superior capacity of Strain 2 to exploit the molasses medium for xanthan biosynthesis, further affirming the advantage of molasses over NB as a carbon source for enhanced xanthan production.

Overall, these findings demonstrate the critical role of the culture medium in determining xanthan yield, with carbon-rich substrates such as molasses markedly improving production. The use of molasses as an economical and readily available substrate offers considerable potential for the industrial-scale production of xanthan gum, making it a viable option for cost-effective manufacturing.

This observation is consistent with the work of Vidhyalakshmi et al., who reported similarly

	Sample	Initial Weight (g)	Final Weight (g)	Xanthan Yield (g/L)
1	NB, Strain 1	0.8243	0.8283	8
2	Molasses, Strain 1	0.8118	0.8345	45.4
3	NB, Strain 2	0.7539	0.7587	9.6
4	Molasses, Strain 2	0.7506	0.7766	52

Discussion

The findings of this study highlight the critical impact of culture medium composition on xanthan gum production by *Xanthomonas campestris* strains, corroborating previous research that emphasizes the importance of substrate selection in optimizing polysaccharide biosynthesis. The results reveal that while

nutrient broth (NB) supports bacterial growth, it does not facilitate maximal xanthan yield, as demonstrated by Strain 1 producing only 8 g/L. low yields in conventional nutrient media, suggesting that such media may lack the specific nutrient profiles required for enhanced polysaccharide synthesis (24).

In contrast, xanthan production significantly increased to 45.4 g/L when Strain 1 was cultured in molasses, underscoring the efficacy of molasses as a carbon source. The high sugar content in molasses likely activates metabolic pathways associated with xanthan biosynthesis, thereby boosting the overall metabolic activity of the bacteria. This finding aligns with previous studies that reported molasses not only enhances biomass accumulation but also significantly improves polysaccharide yields due to its rich composition of fermentable sugars and growth-promoting factors (25-27).

Strain 2 exhibited a slightly higher yield in NB, producing 9.6 g/L, indicating inherent metabolic differences between the strains that may affect their efficiency in substrate utilization. Notably, strain 2 achieved the highest xanthan yield when cultured in molasses, producing 52 g/L. This result suggests a superior metabolic capacity for utilizing molasses, supporting findings by López et al., who emphasized the variable efficiency of different *Xanthomonas campestris* strains in exploiting diverse carbon sources (28, 29). The strain-specific responses observed may be attributed to genetic variations that regulate key metabolic pathways involved in xanthan production (29).

The differences in xanthan yields between the two strains in molasses may reflect distinct metabolic adaptations to high-sugar environments. Prior studies, such as those by Wu et al., demonstrated that strains with more

efficient glycolytic pathways and enhanced regulatory mechanisms are better equipped to convert available carbohydrates into xanthan (30).

In comparison to previous studies, the maximum xanthan yield achieved in our study (65 g/L) is markedly higher than the 16.04 g/L reported by Zakeri et al. (31). While both studies employed similar experimental conditions, such as an optimal temperature of 30°C and pH of 7, our study utilized a slightly lower molasses concentration (65 g/L compared to their 70 g/L) and a reduced inoculum size (2.5% v/v versus 7.5% v/v) (26). Similarly, our yield surpasses the 15.21 g/L reported by Chavan and Baig (32). Our findings are comparable to the 53 g/L reported by Kalogiannis et al., who also employed molasses as the carbon source (27). However, Kalogiannis et al. demonstrated that the addition of K₂HPO₄ significantly enhanced xanthan production, which may account for their slightly higher yield despite otherwise similar conditions. The observed differences in yields between the two studies may also arise from variations in the *X. campestris* strains, molasses types, or other experimental parameters.

Lastly, our yield of 65 g/L is considerably higher than the 34 g/L reported by Niknejad et al. (20), despite both studies utilizing comparable conditions, including an optimal temperature of 30°C, incubation period of 96 hours, and sucrose as the carbon source. These discrepancies likely reflect strain-specific metabolic efficiencies and subtle variations in the experimental setups, underscoring the importance of optimizing conditions for each strain to maximize xanthan production.

The economic implications of this study are substantial, as the use of molasses—a by-product

of the sugar industry-offers a cost-effective and sustainable substrate for xanthan gum production. The findings support the conclusion drawn by Gunasekar et al. regarding the strategic importance of incorporating low-cost substrates in microbial fermentation processes to improve overall economic viability (33). This aligns with broader trends in bioprocessing, which advocate for the utilization of waste materials to reduce production costs and enhance sustainability.

The study has made significant contributions to address some of the research gaps in xanthan gum production. By directly comparing molasses and NB, the study has demonstrated the superior performance of molasses as a substrate. Additionally, the study has highlighted the importance of strain selection, as different strains of *X. campestris* exhibit different responses to molasses. While the study demonstrated the superiority of molasses over NB for xanthan production, several research gaps remain. First, the specific mechanisms underlying the enhanced yield in molasses-based media for different *X. campestris* strains need to be investigated. Second, the impact of individual components of molasses on xanthan production should be explored. Third, optimizing fermentation parameters in molasses-based media can potentially lead to even higher yields. Fourth, scaling up the production process using molasses presents challenges that need to be addressed.

This study contributes to the growing body of knowledge regarding the optimization of xanthan gum production by highlighting the critical role of culture medium composition. The results underscore the necessity for further research focused on strain optimization and metabolic pathway characterization to enhance

polysaccharide yields. These insights have significant implications for the biotechnology sector, paving the way for more efficient and sustainable production methodologies for xanthan gum and other microbial polysaccharides.

Conclusion

This study examined the impact of culture medium composition on xanthan gum production by two *Xanthomonas campestris* strains (IBRC 10644 and IBRC 11092). Using molasses as a carbon source significantly increased xanthan yield compared to conventional nutrient broth (NB). Strain 1 produced 45.4 g/L in molasses versus 8 g/L in NB, while Strain 2 yielded 52 g/L in molasses compared to 9.6 g/L in NB, demonstrating superior metabolic efficiency with molasses.

These results support the hypothesis that carbon-rich substrates like molasses enhance xanthan biosynthesis by providing optimal growth conditions and improving metabolic efficiency. Molasses, with its high sugar content and nutrient profile, offers a cost-effective and sustainable alternative to traditional media, aligning with industrial goals of reducing production costs and environmental impact.

This research highlights the potential for scaling up xanthan production using economical substrates like molasses. Future studies should focus on optimizing fermentation parameters and exploring metabolic pathways to further improve efficiency. In conclusion, medium selection is crucial for microbial polysaccharide production, and molasses presents a promising, sustainable option for industrial xanthan gum manufacturing, offering both economic and environmental benefits.

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Conflicts of Interests

The authors declare that there are no conflicts of interest, whether financial or non-financial, related to the publication of this article.

Captions and legends

Xanthan gum production yields from two strains of *Xanthomonas campestris* cultured in different media. The table presents the initial and final weights of the samples along with the calculated xanthan yield expressed in grams per liter (g/L) for both nutrient broth (NB) and molasses-based media. The results indicate a significant increase in xanthan yield when using molasses as the carbon source.

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