International Journal of Innovative Research and Scientific Studies, 8(2) 2025, pages: 2530-2542

IJIRSS
International Journal of Innovative Research & Scientific Studies

ISSN: 2617-6548

URL: www.ijirss.com

Improved xanthan gum production by a newly *Xanthomonas melonis*: A promising biopolymer for drilling fluids

Ahmad Marasabessy[1*], Anis Herliyati Mahsunah[2], Dyah Noor Hidayati[3], Panca Wahyudi Soekarno[4], Edy Marwanta[5]

[1]*Directorate of Utilization of Research and Innovation by Industry, National Research and Innovation Agency (BRIN), Republic of Indonesia, Jakarta Pusat 10340, Indonesia.*
[2,3]*Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Republic of Indonesia, KST Soekarno, Cibinong 16911, Jawa Barat, Indonesia.*
[4]*Research Center for Process and Manufacturing Industry Technology, National Research and Innovation Agency (BRIN), Republic of Indonesia, KST B.J. Habibie, Tangerang Selatan 15314, Banten, Indonesia.*
[5]*Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), Republic of Indonesia, KST Soekarno, Cibinong 16911, Indonesia.*

Corresponding author: Ahmad Marasabessy (*Email: ahma011@brin.go.id*)

Abstract

This study investigates xanthan gum production using bacterial strains isolated from plants. Bacteria were screened from local plant sources, fermentation conditions were optimized, and the viscosity of the produced xanthan gum was evaluated for oil drilling applications. A total of 27 bacterial strains were isolated from yellow pumpkin leaves, wild grape leaves, and the bark resin of the red water guava tree. Four strains with high gum viscosity were identified: Xanthomonas melonis LBU-4, X. melonis LBU-8, X. sacchari AGR-5, and Sphingomonas panni LBU-7. Shaken flask fermentation using different carbon sources showed that X. melonis LBU-4, grown in a sucrose-rich medium, produced the highest xanthan gum yield (22.8 g/l) with high viscosity after 120 hours of cultivation. FTIR spectroscopy confirmed the structural similarity between the produced xanthan gum and the commercial xanthan gum. Viscosity tests of the produced xanthan gum comply with American Petroleum Institute (API) 13A standards for drilling-grade xanthan gum. These findings highlight X. melonis LBU-4 as a promising candidate for industrial-scale xanthan gum production.

Keywords: Drilling fluids, Pumpkins, Sucrose, Xanthan gum, Xanthomonas melonis.

DOI: 10.53894/ijirss.v8i2.5732
Funding: This work was supported and funded by the National Research and Innovation Agency (BRIN), Republic of Indonesia.
History: Received: 7 February 2025 / **Revised:** 7 March 2025 / **Accepted:** 13 March 2025 / **Published:** 27 March 2025

Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.
Transparency: The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.
Acknowledgements: The authors acknowledge the National Research and Innovation Agency (BRIN) for funding this research project. The authors acknowledge the facilities, scientific and technical support from Zoology Characterization Laboratories, National Research and Innovation Agency through E-Layanan Sains, Badan Riset dan Inovasi Nasional (BRIN), for conducting SEM analysis.
Publisher: Innovative Research Publishing

1. Introduction

Xanthan gum is a bacterial polysaccharide that is widely utilized as a stabilizer, food additive, and thickening agent. It is an important biopolymer with high commercial value both in domestic and foreign markets due to its extensive use in various food products such as drinks, dairy items, salad dressings, sauces, and confectioneries [1, 2]. It is also an excellent additive in drilling fluids for oil and gas exploration due to its pseudo-plastic feature and thermal stability, as well as its favorable salt compatibility [3, 4]. The global market for xanthan gum has been triggered by the increased use of this gum as a less expensive substitute for guar gum [5, 6].

The value of the worldwide xanthan gum market increased from USD 270 million in 2006 to USD 400 million in 2015. The market value surged to over USD 650 million in 2021, and from this point until 2031, it is expected to expand at a compound annual growth rate (CAGR) of 5.9% [1, 3]. The market is predicted to grow to USD 1.9 billion by 2031 [1].

Xanthan gum is produced through fermentation by bacteria, notably within the genus Xanthomonas [7]. The bacterial strains, medium compositions, and other environmental conditions affect the physical and chemical properties of the resulting xanthan gum molecule [5, 8]. The primary carbon sources required as substrates for the synthesis of xanthan gum include glucose, sucrose, and certain other types of carbohydrates like maltose and dextrin [8, 9]. Other crucial nutrients for the synthesis of xanthan gum include both organic and inorganic nitrogen. Additionally, phosphate, magnesium, and other minerals are required [8, 10].

Bacteria excreting this natural gum are usually found in the rotten leaves and fruits of some agricultural plants that suffer from black rot symptoms, such as cabbage (Brassica oleracea, L.), yellow pumpkin (Cucurbita moschata, L.), and grapes [11-13]. This bacterium is aerobic and rod-shaped with polar flagella, measuring 0.4 to 0.7 µm in width and 0.7 to 1.8 µm in length [14]. The cells are surrounded by capsules and do not form spores. A colony of this bacterium in agar media has a yellowish-white color, is round-shaped, and has a smooth and slimy edge surface. Almost all strains of Xanthomonas are monotrichous and gram-negative, causing necrosis (local tissue death) in monocotyledonous and dicotyledonous plants [8].

The work reported here aimed to obtain a superior bacterial species from local sources to be used in xanthan gum production. The local sources were the decaying leaves of pumpkin and grapes, as well as the bark resin of red water guava. After the identification of some selected bacteria through a molecular approach, the optimization of the fermentation medium using local raw materials as substrates for laboratory-scale fermentation was conducted. Xanthan gum production was performed on a limited scale by using the best species in order to obtain purified gum for FTIR spectroscopy analysis and assessment of its potential application as a drilling fluid material in fossil oil or energy explorations.

2. Literature Review

2.1. Microbial Gums

Polysaccharide gums can be produced from animals, plants, fungi, and microorganisms. Their use in the food, biotechnology, and medical industries is growing due to their structural diversity, unique rheological properties, biodegradability, and wide availability [8, 15]. Plant gums typically have more complex polysaccharide structures [15]. In contrast, microbial gums such as xanthan gum are extracellular biopolymers produced by microorganisms, consisting of simple sugar units such as glucose, mannose, and fructose. These microbial gums function as stabilizers and gelling agents, improving the rheological properties of various products [3, 16, 17].

2.2. Xanthan Gum Producing Bacterial Strains

Xanthan gum, an extracellular polysaccharide commonly produced by *X. campestris*, is widely used in the food, pharmaceutical, and oil recovery industries. Its structure consists of a β-D-glucose backbone with charged trisaccharide side chains, including glucuronic acid and mannose residues Casas et al. [5]. Gumus et al. [11] studied xanthan gum production by different *Xanthomonas* strains. The research evaluated productivity and viscosity characteristics, comparing novel isolates with the standard *X. campestris* NRRL B-1459. Notably, *X. arbicola* pv. *Juglandis* exhibited the highest productivity (8.22 g/l), demonstrating its potential for industrial applications. However, the study primarily focuses on glucose as the carbon source, overlooking alternatives like agricultural waste, which could enhance yield and reduce cost.

Dai et al. [18] present a novel approach to reducing ethanol use in xanthan gum production by genetically modifying *X. campestris* CGMCC15155. By knocking out the *pigA* gene responsible for xanthomonadin pigment synthesis and inserting the *Vitreoscilla globin* (*vgb*) gene, the researchers obtained a 133.3% reduction in ethanol consumption for purification while maintaining xanthan yield at wild-type levels.

To the best of our knowledge, there are no research articles reporting the use of *X. melonis* for xanthan gum production, either at the research scale or the production scale.

2.3. Xanthan Gum Production Technology

Casas et al. [5] reported factors affecting the molecular structure and rheological properties of xanthan gum. Higher temperatures (e.g., 34°C) lead to lower molecular weight and lower acetate and pyruvate content, whereas lower temperatures (e.g., 25°C) result in xanthan with higher molecular weight and viscosity. A peak pyruvate content is observed around 27–28°C. The nitrogen source affects pyruvilation, with organic nitrogen sources yielding higher pyruvate content. Higher nitrogen concentrations generally decrease pyruvilation but do not significantly affect molecular weight. Adequate oxygen levels are necessary for optimal xanthan production. Studies show that oxygen limitations reduce molecular weight, while excessive stirring damages cells, lowering xanthan yield. A stirrer speed of the fermenter around 500 rpm optimizes production. Longer fermentation times increase molecular weight, acetate, and pyruvate content, although some researchers argue that pyruvate content stabilizes in the stationary phase.

Some studies explore various approaches to enhancing xanthan gum production and its integration into biorefinery systems. Soltaninejad et al. [19] propose a biorefinery model that utilizes potato crop residues to produce xanthan gum, bioethanol, and biogas. Their research demonstrates the effectiveness of organosolv pretreatment in increasing glucose yield for fermentation, which, in turn, improves xanthan gum production.

Saydam et al. [20] examine the techno-economic aspects of xanthan gum production from sugar beet molasses using process simulation. The study employs the Taguchi method for sensitivity analysis, revealing that factors such as raw material price and transportation costs significantly influence profitability.

3. Materials and Methods

3.1. Materials

The rotten leaves of yellow pumpkin (Cucurbita moschata, L; Family of Cucurbitaceae; local name: waluh) and the rotten leaves of wild grapes (unknown name) were collected from Bogor, Indonesia (Figure 1). The bark resin of the red water guava tree (Syzygium malaccense, L; Family of Myrtaceae; local name: jambu bol merah, jambu air jamaika) was collected from a tree of this plant located in the same area mentioned above (Figure 1).

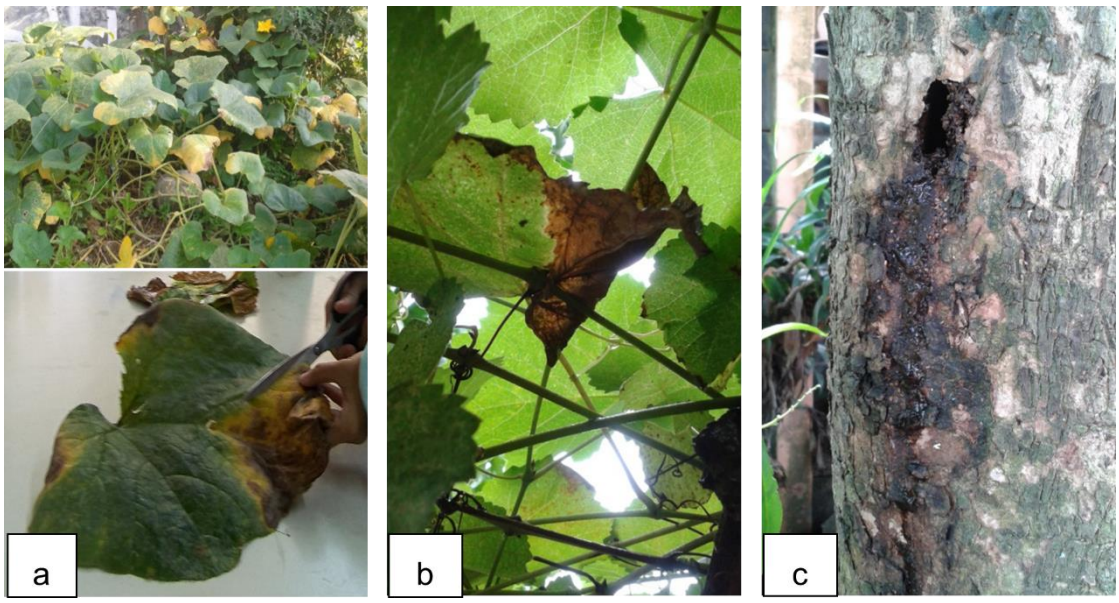

Figure 1.
The plant samples used in this study are: (a) the rotten leaves of yellow pumpkin; (b) the rotten leaves of wild grape; and (c) the bark containing resin of red water guava tree.

3.2. Isolation and Screening of Xanthan Gum-Producing Bacteria

The isolation of bacteria from the pumpkin's rotten leaf, the grape's rotten leaf, and the bark resin of the red water guava was done individually according to the following steps: The plant leaves were cleaned thoroughly with sterile distilled water. The infected parts of the leaves were taken and cut into small pieces with disinfected scissors (70% alcohol disinfection). About 1.0 g of the leaf pieces or the resin was placed into a sterile plastic bag, mixed with 10 ml of sterile water, and then placed inside a stomacher blender to crush and homogenize the sample material. One ml of the liquid portion was pipetted out and underwent serial dilution with 0.9% sodium chloride solution until a 10–8 dilution was achieved. After that, 0.1 ml of liquid from each dilution, starting from 10-3 to 10-8 dilutions, was spread on a Kings B agar plate (Himedia) in a petri

dish. Each dilution was inoculated in duplicate. The plates were incubated at 35 °C for 48 h. The well-separated single colony showing visual morphology identical to the Xanthomonas colony was picked up with an inoculation needle and sub-cultured using a four-way streak method on a fresh Kings B agar plate for strain purification. From the plates, a total of 27 bacterial colonies were selected. Each selected colony was subcultured on YM agar slant (Yeast extract-maltose agar, Himedia) prior to use.

3.3. Inoculum Preparation

The inoculum was made by inoculating a 24-hour-old culture of agar into 100 ml of liquid medium of YM Broth (Yeast Extract-Maltose Broth, Himedia) at pH 7.0 in a 500 ml flask. The cultures were shaken at 120 rpm and 28 °C for 24 h in the Innova 44 Orbital Shaker (USA). The production medium was inoculated with this inoculum (10% v/v) in order to produce xanthan gum.

3.4. Investigation of the Xanthan Gum Production of the Selected Bacteria

The aim was to choose species among the selected bacteria able to produce viscous gum in the fermentation broth. For a single bacterium, 10 ml of inoculum propagated in YM broth was transferred to 90 ml of production medium in a 250-ml Erlenmeyer flask. The basic production medium (BPM) comprises (per liter): 30.0 g glucose, 0.935 g glutamic acid, 2.31 g citric acid, 5.0 g KH_2PO_4, 0.2 g $MgSO_4$, 0.02 g $ZnSO_4$, 0.006 g boric acid, and distilled water up to 1000 ml, pH 7.0. The fermentation was run for 120 hours at 28 °C and shaken at 160 rpm using the Innova 44 Orbital Shaker. The bacteria exhibiting high gum viscosity and gum weight in the fermentation broth were identified genetically to reveal their homology relationship for species name determination. Later, the identified bacterial species were investigated for their gum production capability in different carbon sources.

3.5. Viscosity Measurement

Viscosity was measured with a Brookfield viscometer type DV-E using spindles 61, 62, 63, or 64 at 30 rpm and at 25–28 °C. Viscosity was expressed in centipoise (cP).

3.6. Identification of Isolated Bacterium

The isolation of bacterial DNA was performed using a FastPrep@DNA kit (MP Biomedicals, USA). The amplification of the 16S rRNA sequence was carried out by PCR with the primers 27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-GGATACCTTGTTACGA CTT-3', using the cycling conditions as follows: 94 °C for 3 minutes, then 30 cycles of 1 minute at 94 °C, 1 minute at 55 °C, and 2 minutes at 72 °C, with a final extension for 10 minutes. The amplicon was sent for purification and sequencing at 1st Base (Malaysia). The raw obtained sequences were edited using ATGC software (Montpellier Bioinformatics Platform, Institut Français de Bioinformatique & France Génomique), and the results were matched to the bacterial genes database in the National Center for Biotechnology Information (NCBI) GenBank (http://www.ncbi.nlm.nih.gov/) using BLAST (Basic Local Alignment Search Tool) (NCBI, Rockville Pike, Bethesda, MD, USA). The evolutionary history was concluded as has been explained [21, 22]. The alignment of all sequences was done by Clustal W (Arizona State University, Tempe, AZ, USA) using MEGA 11, and all ambiguous sites were trimmed for each pair of sequences. Finally, the Neighbour-Joining phylogenetic trees were built using MEGA software 11 [23].

3.7. Gum Production by Different Bacterial Strains Using Various Carbon Sources

The medium used for gum production using various carbon sources was BPM medium, in which only glucose was replaced by another sugar or carbohydrate of the same weight. The other sugars used to replace glucose in BPM medium were lactose (food-grade), sucrose (food-grade), maltodextrin (food-grade), palm sugar (food-grade), cassava starch (food-grade), or cane molasses (feed-grade). The experiment was conducted in duplicate. The fermentation operational conditions were as follows: 10 ml of inoculum in 90 ml of production medium, a temperature of 28 ºC, a shaking speed of 160 rpm, and a fermentation time of 120 h.

3.8. Gum Recovery and Weight Measurement

The harvested fermentation broth was pasteurized in an autoclave at 90 °C for 25 min, and then centrifuged at 6,500×g for 30 minutes at 4–10 °C to remove the cells. The separated supernatant was used to precipitate the xanthan gum by mixing with isopropanol in a 1:2 (v/v) ratio. The gum was then separated by filtration forced by a vacuum pump. The wet gum was dried at 45–55 °C in a vacuum oven for 3–5 hours until a constant weight was reached. After that, its dry weight was measured [11].

3.9. FTIR Spectroscopy Analysis

Fourier-Transform Infrared Spectroscopy/FTIR used was a Bruker-Tensor II Spectrometer (Bruker Optics) using a frequency range of 4000–500 cm^{-1}, a number of scans of 45 seconds, and a resolution of 4.0 cm^{-1}.

3.10. Assessment of the Viscosity Characteristics of Xanthan Gum

The viscosity characteristics of xanthan gum were assessed according to the standards of API Spec. 13A/ISO 13500 [24]. For this purpose, a solution of xanthan gum in seawater was prepared from a raw sea salt solution.

3.11. Miscellaneous Analyses

The number of colonies forming units (cfu) was determined by the serial dilution method, as described elsewhere, by using King's B medium plates. A scanning electron micrograph (SEM) was conducted using the JSM-IT200 (JEOL, Japan).

4. Results

4.1. Isolation And Screening of Xanthan Gum-Producing Bacteria

After 48 hours of incubation, 27 well-separated bacterial colonies were isolated from the three plant samples grown on King's B agar plates: 11 from pumpkins (LBU), 7 from grapes (AGR), and 9 from red water guava (JMB). The macroscopic characteristics of these colonies are detailed in Table 1.

Table 1.
Macroscopic characteristics of 27 colonies isolated from King's B agar plate cultures.

Colony	Colony Color	Colony Configuration	Margin Shape	Edge Elevation
LBU 1	Yellow	Round	Smooth	Convex
LBU 2	Yellow	Round	Smooth	Raised
LBU 3	Yellow	Irregular		Flat
LBU 4	Yellow	Round	Smooth	Raised
LBU 5	White	Round	Smooth	Raised
LBU 6	Yellow	Round with raised margin	Smooth	Convex
LBU 7	Yellow	Round	Smooth	Flat
LBU 8	Yellow	Round with raised margin	Smooth	Convex
LBU 9	Pale yellow	Round with raised margin	Smooth	Raised
LBU 10	White	Round	Smooth	Raised
LBU 11	Creamy white	Round	Wavy	Raised
AGR 1	Yellow	Round	Smooth	Convex
AGR 2	Creamy white	Round	Smooth	Raised
AGR 3	Yellow	Round	Smooth	Convex
AGR 4	Yellow	Round with raised margin	Smooth	Convex
AGR 5	Yellow	Round	Smooth	Convex
AGR 6	Yellow	Round	Smooth	Flat
AGR 7	Yellow	Round	Smooth	Convex
JMB 1	White	Concentric	Smooth	Convex
JMB 2	White	Round with raised margin	Wavy	Convex
JMB 3	Yellow	Round With Raised Margin	Wavy	Raised
JMB 4	Yellow	Round with raised margin	Wavy	Raised
JMB 5	Yellow	Round	Smooth	Convex
JMB 6	White	Round	Smooth	Raised
JMB 7	White	Round with raised margin	Smooth	Flat
JMB 8	White	Round with raised margin	Lobate	Raised
JMB 9	White	Round with raised margin	Wavy	Raised

Fermentation tests by using these 27 colonies to screen xanthan gum-producing bacteria resulted in four selected bacterial colonies exhibiting relatively high gum viscosity, ranging from 620 to 7860 cP, and gum weights from 5.7 to 14.3 g/l (detailed data not presented). These colonies, named LBU-4, LBU-7, LBU-8 (isolated from pumpkin), and AGR-5 (isolated from grape), demonstrate potential as xanthan gum producers.

4.2. Genetic Identification of Bacteria

The data of 16s rRNA sequence data of the four bacterial isolates in this research were submitted to GenBank with accession numbers ON514275, OP994059, OP999124, and OP999125, and sequence data of the closest related species were acquired from the same database. These data sets were used to build a phylogenetic tree with the neighbor-joining method and to analyze the phylogenetic relationship. The similarity level analysis based on the 16S rRNA sequence indicated that bacteria LBU-4, LBU-8, and AGR 5 are closely related to the members of *Xanthomonas*, while LBU-7 is closely related to the members of *Sphingomonas* (Figure 2). Based on these data, the assignments of bacteria LBU-4, LBU-8, AGR-5, and LBU-7 were proposed as *X. melonis* LBU-4, *X. melonis* LBU-8, *X. sacchari* AGR-5, and *S.panni* LBU-7, respectively. *X. melonis* LBU-4, *X. melonis* LBU-8, *X. sacchari* AGR-5, and *S.panni* LBU-7 will be written as *Xm*LBU-4, *Xm*LBU-8, *Xs*AGR-5, and *Sp*LBU-7, respectively.

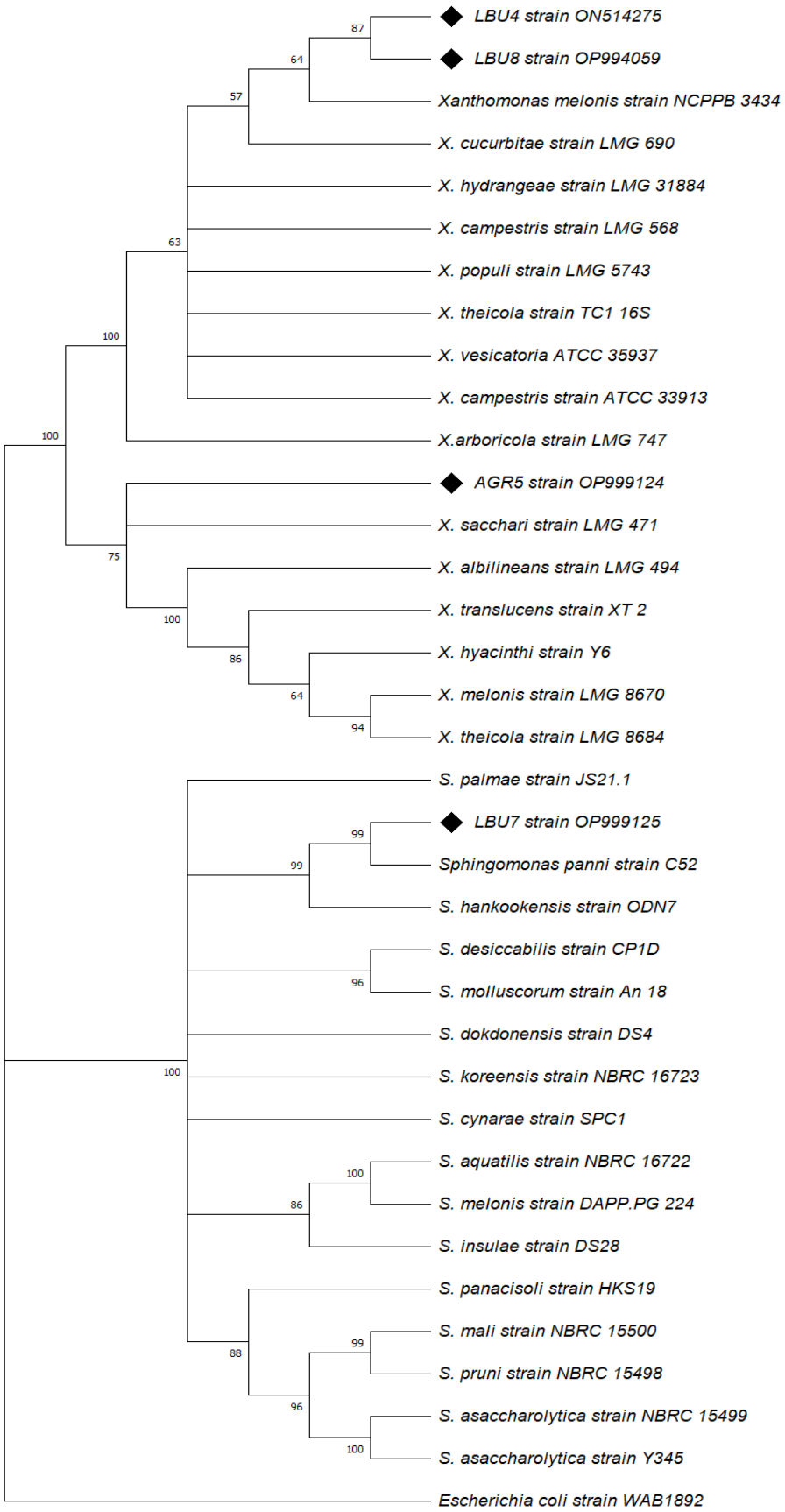

Figure 2.
Phylogenetic tree built from 16S rRNA sequence of Xanthomonas spp. and S.panni isolated in this study (LBU-4, LBU-8, AGR-5, LBU-7), and other species of Xanthomonads and Sphingomonas obtained from GenBank. The tree was built using MEGA software (version 11) by neighbour-joining (1000 bootstrap repetitions); only bootstrap values greater than 50% are indicated. The p-distance method was used to calculate the evolutionary distances in units of the number of base changes per position. Escherichia coli strain WAB 1892 was used as the out-group taxon.

4.3. Xanthan Gum Production by Different Bacterial Strains Using Various Carbon Sources

To identify the optimal bacterial strain and carbon source for future xanthan gum production studies, fermentation broth viscosity and gum titer were considered the primary selection criteria. Thus, selecting a suitable carbon source and a bacterial strain with high gum viscosity and titer was crucial. In this study, seven carbon sources were tested: glucose, lactose, sucrose, maltodextrin, palm sugar, cassava starch, and molasses. Additionally, four selected bacterial strains (*X. melonis* LBU-4, *S. panni* LBU-7, *X. melonis* LBU-8, and *X. sacchari* AGR-5) were evaluated. Figure 3 and Figure 4 show the fermentation broth viscosity and gum yield, respectively, for each strain grown in media containing a single carbon source.

From Figure 3**,** *Xm*LBU-4 produced significantly high viscosity with sucrose (15,800 cP), palm sugar (12,660 cP), and cassava starch (12,420 cP) compared to molasses (7,470 cP), glucose (3,809 cP), malto-dextrin (1,188 cP), and lactose (150 cP). Similarly, *Xm*LBU-8 generated high viscosity with sucrose (13,090 cP), cassava starch (12,950 cP), and palm sugar (12,030 cP) compared to glucose (7,860 cP), molasses (7,020 cP), malto-dextrin (1,346 cP), and lactose (149). Figure 3 also confirms that *Sp*LBU-7 and *Xs*AGR-5 generated low viscosity in all carbon substrates examined. In summary, sucrose, palm sugar, and cassava starch are the most suitable carbon sources for both *Xm*LBU-4 and *Xm*LBU-8 to produce viscous exopolysaccharides, while molasses, glucose, and malto-dextrin are less suitable. In contrast, lactose practically did not influence the formation of viscous exopolysaccharides in all bacterial strains examined.

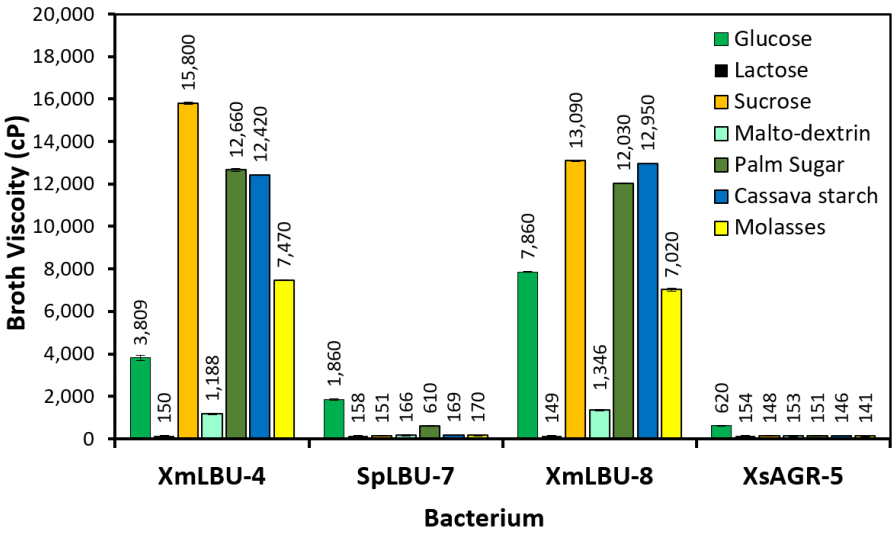

Figure 3.
Fermentation broth viscosity in different carbon sources produced by the four identified bacteria (XmLBU-4 is X. melonis LBU-4; SpLBU-7 is S. panni LBU-7; XmLBU-8 is X. melonis LBU-8; XsAGR-5 is X. sacchari AGR-5).

In terms of gum weight, as shown in Figure 4, XmLBU-4 produced a gum titer of more than 20.0 g/l in the medium with sucrose (22.8 g/l), palm sugar (20.8 g/l), and cassava starch (20.1 g/l). Similarly, XmLBU-8 produced a closely comparable gum quantity in the medium containing sucrose (22.3 g/l), palm sugar (21.8 g/l), and cassava starch (21.7 g/l). However, both bacteria were unable to synthesize viscous exopolysaccharides from lactose.

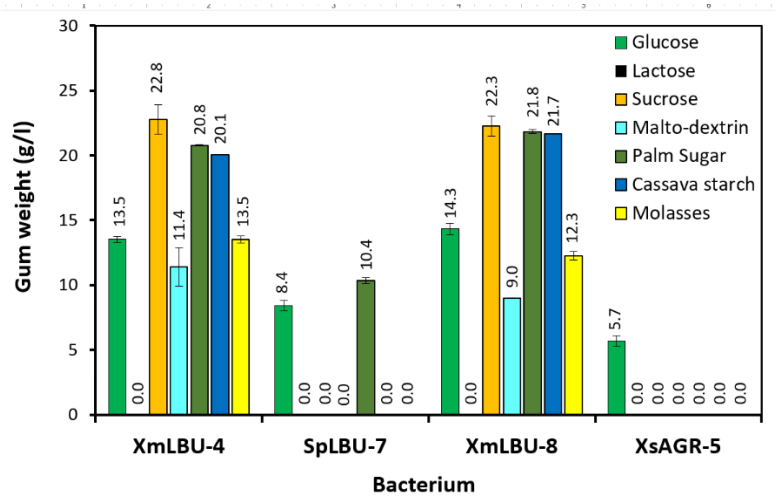

Figure 4.
Gum weight in different carbon sources produced by the four identified bacteria (XmLBU-4 is Xanthomonas melonis LBU-4; SpLBU-7 is Spingomonas panni LBU-7; XmLBU-8 is Xanthomonas melonis LBU-8; XsAGR-5 is Xanthomonas sacchari AGR-5).

Figure 3 demonstrates that both *Sp*LBU-7 and *Xs*AGR-5 did not produce significant viscosity in most of the carbon substrates studied and were excluded as the strain of choice for further experiments. Hence, the final bacterial strain to be chosen relied on the viscosity and gum weight profiles of *Xm*LBU-4 and *Xm*LBU-8. Both *Xm*LBU-4 and *Xm*LBU-8 produced high gum weight in the medium containing sucrose, palm sugar, and cassava starch (Figure 4); however, their corresponding broth viscosities revealed that *Xm*LBU-4 produced the highest viscosity in sucrose media (Figure 3). Consequently, *Xm*LBU-4 was chosen as the only experimental bacterium used in further experiments. In addition, sucrose was used as the only experimental carbon source. The isolate of *X.melonis* LBU-4 (*XmLBU*-4) has been deposited in the Indonesian Culture Collection (InaCC) under accession number InaCC B 1642.

4.4. SEM Feature of X. Melonis LBU-4

Figure 5 shows the SEM image of the 48-hour-old bacterial cells of XmLBU-4 grown on King's agar medium. The cells are rod-shaped, with a size of 0.5 to 0.7 µm wide and 1.0 to 1.5 µm long. The cells are still young, so slimy gum has not yet been excreted outside the cells. In addition, the cell flagellum is practically not visible in the image. The chemicals used in sample preparations for SEM analysis might have a destructive effect on the bacterial flagella.

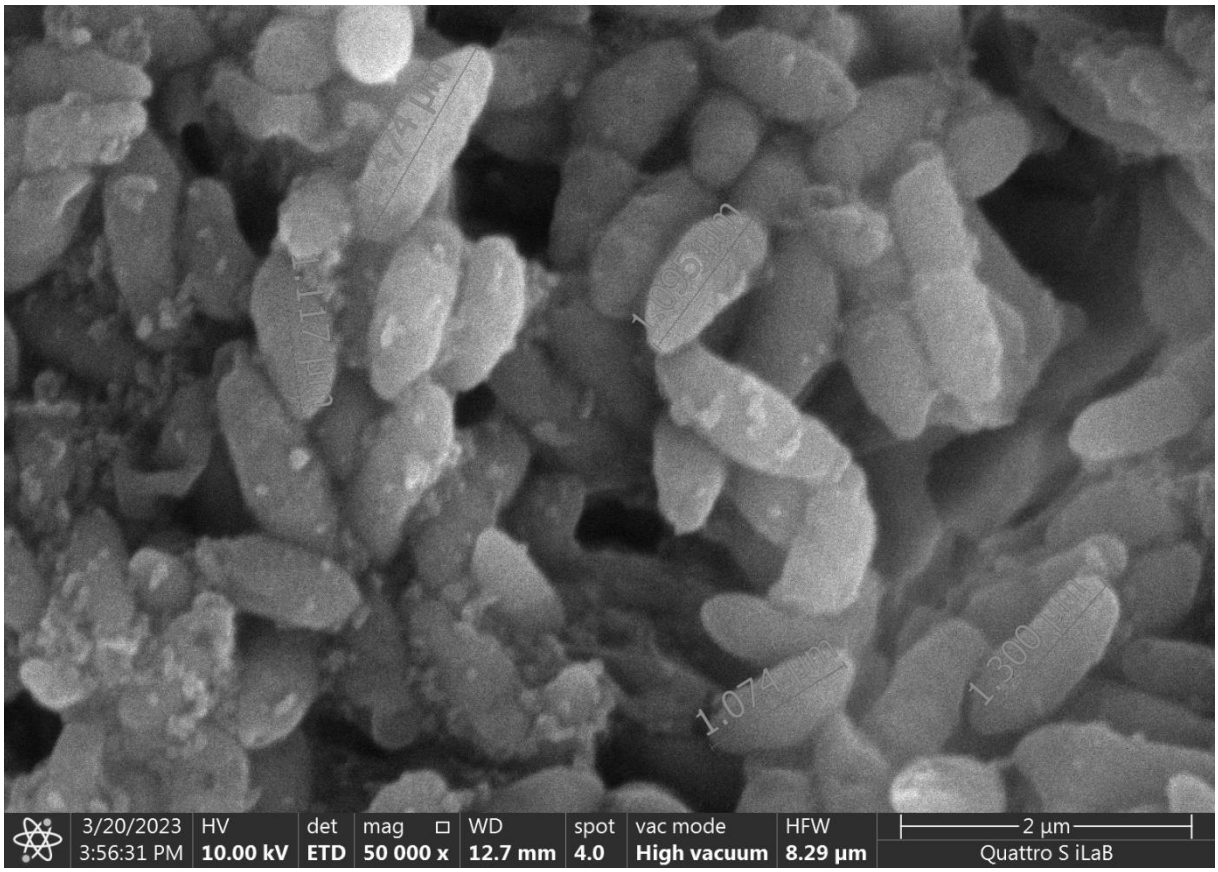

Figure 5.
Scanning electron microphotographs of X. melonis LBU-4 at 50,000× magnification.

4.5. Gum Production for FTIR Spectroscopy and Field Performance Property Tests

The additional shaken flask fermentation with a total volume of 4,000 ml was performed using *Xm*LBU-4 to obtain the required quantity of gum powder for FTIR spectroscopy analysis and field performance property tests. As a carbon source, sucrose was used to replace glucose at the same concentration (30 g/l). The other ingredients and fermentation conditions were kept the same.

To obtain gum powder with less impurity, in the extraction process, an additional step of gum precipitation is carried out by re-dissolving the wet gum obtained from the first extraction using distilled water in an equal volume of its corresponding broth culture, followed by precipitation with the same procedure described in Gum recovery and weight measurement to obtain dried gum above. The same purification procedure for commercial food-grade xanthan gum was also performed to serve as a comparative sample in FTIR spectroscopy analysis.

The aim of the FTIR spectroscopy analysis was to confirm the molecular structure of xanthan gum produced by the bacterium from this research and to compare it to spectra obtained from commercial xanthan gum. Xanthan gum is a polysaccharide whose backbone consists mainly of repeating β-D-glucose units connected by β-1,4 glycosidic bonds, with side chains of D-mannose and D-glucuronic acid. These side chains are linked to the backbone through β-1,2 and β-1,3 glycosidic bonds, respectively. The molar ratio of glucose, mannose, and glucuronic acid is 2:2:1 [10, 24]. An acetyl group is attached to the D-mannose unit linked to the main chain, while a pyruvic acid residue is attached to the terminal D-mannose unit [3, 10].

The comparison of FTIR spectra between the gum produced by *Xm*LBU-4 and the purified commercial xanthan gum is presented in Figure 6. It has been proven that the chemical structure of the gum produced by *Xm*LBU-4 is highly identical to that of commercial xanthan gum. The analyzed region encompasses all spectral bands within the range of the wave numbers 500 and 4000 cm^{-1}. The most important bands were: around 3275 cm^{-1} (O-H bonds), around 2927 cm^{-1} (C-H bonds of CH2 groups), around 1720 cm^{-1} (small band, attributed to acetyl groups), around 1602 cm^{-1} (asymmetric stretching of COO^-), around 1406 cm^{-1} (symmetric stretching of $–COO^-$), around 1247 cm^{-1} (C-H bonds of saccharides), and around 1024 cm^{-1} (fingerprints). Faria et al. [25] reported similar FTIR spectra of xanthan gum that was produced by *X.campestris* pv. *campestris* NRRL B-1459 utilizing diluted sugar cane broth.

Based on these findings, it can be confirmed that the gum produced by XmLBU-4 is the xanthan gum substance. To give a specific identity to the product for field testing, we have named our xanthan gum powder product XCD BRIN.

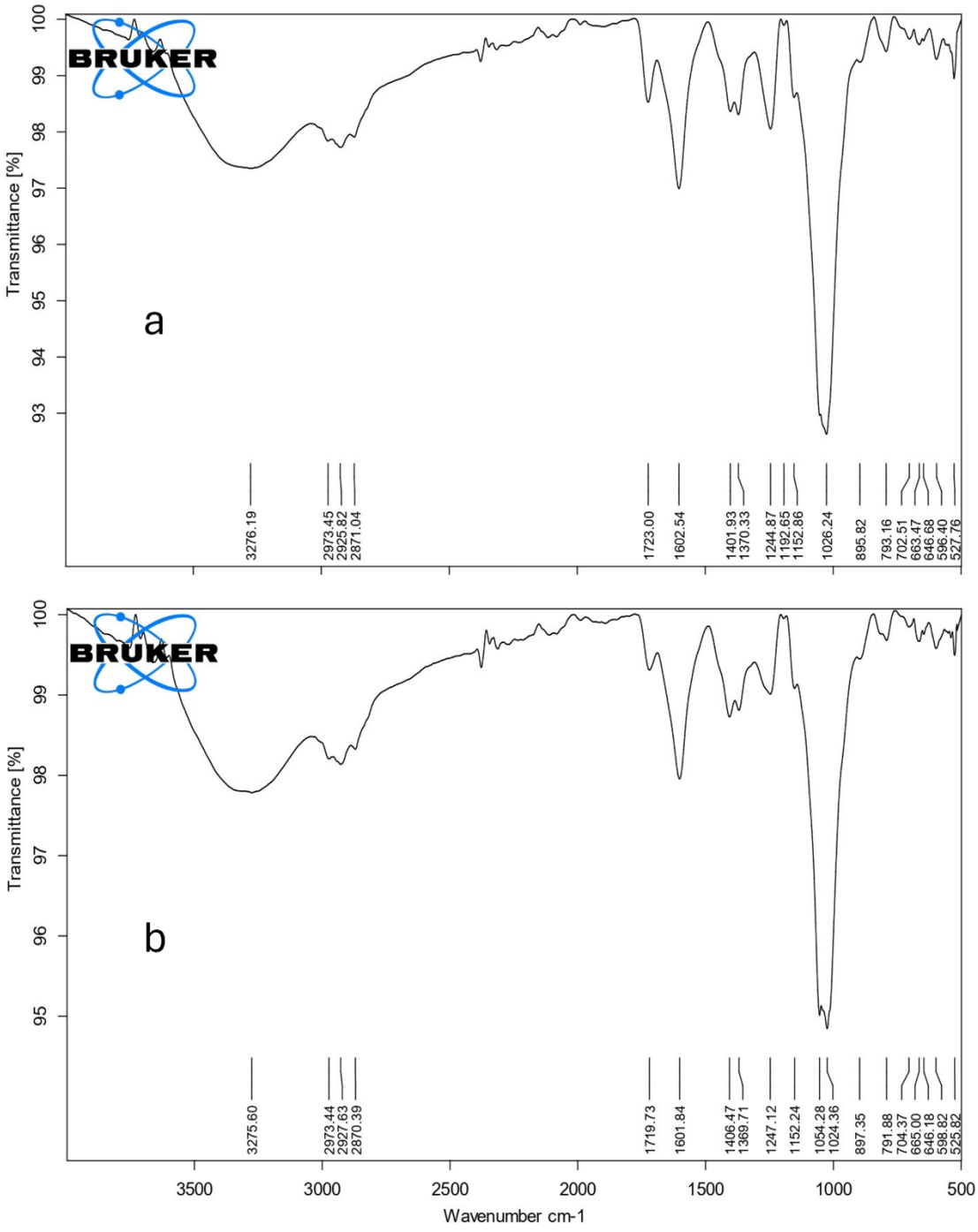

Figure 6.
Comparison of the FTIR spectra between xanthan gum from XmLBU-4 (a) and commercial xanthan gum (b).

4.6. The Assessment of Xanthan Gum Quality

The viscosity characteristics of xanthan gum were assessed to evaluate whether XCD BRIN could be used for industrial applications, specifically as an additive in oil drilling fluid. A commercial drilling-grade xanthan gum obtained from the market was used as a reference sample. It is demonstrated that XCD BRIN fulfilled all property parameters required by the

API (American Petroleum Institute) Standard, with performance highly comparable to commercial drilling-grade xanthan gum (Table 2).

Table 2.
Viscosity characteristics of the XCD BRIN in comparison to those of drilling-grade xanthan gum. The rheology test used Fan Model 35 A-1 Spring, according to API Spec. 13A/ISO 13500 Standard.

No	Requirement	(API Spec. 13A/ISO 13500 Standard) [26]	Our results (XCD BRIN)	Drilling grade Xanthan gum
1	Starch, guar, or their derivatives	Absent	Absent	Absent
2	Moisture content	Maximum 13%	8.74%	11.57%
3	Screen analysis			
	a) Less than 425 µm	Minimum 95%	Minimum 95%	Minimum 95%
	b) Less than 75 µm	Maximum 50%	Maximum 50%	Maximum 50%
4	Viscosity			
	a) Rotational viscometer, 300 r/min	Minimum 11 cP (Minimum 55 dial reading)	14.2 cP (71 reading)	14.6 cP (73 reading)
	b) Rotational viscometer, 6 r/min	Minimum 180 cP (Minimum 18 dial reading)	270 cP (27 dial reading)	230 cP (23 dial reading)
	c) Rotational viscometer, 3 r/min	Minimum 320 cP (Minimum 16 dial reading)	440 cP (22 dial reading)	400 cP (20 dial reading)
	d) Brookfield LV, 1,5 r/min	Minimum 1950 cP	2084 cP	2547

5. Discussion

5.1. Isolation and Screening of Xanthan Gum-Producing Bacteria

Our experiments have shown that the potential bacterial strains for xanthan gum production were isolated from bacteria-infected pumpkin leaves, namely XmLBU-4 and XmLBU-8. These strains exhibit superiority in xanthan gum production from media containing sucrose, palm sugar, and cassava starch.

In previous studies, xanthan gum was produced in a glucose medium by some other strains of Xanthomonas spp. isolated from different kinds of plants, such as X. arboricola pv. juglandis from walnut (Juglans regia L.), X. axonopodis pv. vesicatoria from pepper (Capsicum annuum L.), and X. axonopodis pv. begonia from begonia (Begonia X tuberhybrida) and anthurium (Anthurium andraeanum) [11].

A screening of *Xanthomonas* spp. from infected Brassicaceae crops for xanthan gum production showed that out of 411 isolates, ten were selected as potential strains of xanthan gum producers [27].

Our report, as well as the previous reports, indicate a large distribution of *Xanthomonas* strains living in various plant species as well as their tangible differences in the ability to convert different carbohydrates into xanthan gum molecule (s).

5.2. Xanthan Gum Production by Different Bacterial Strains Using Various Carbon Sources

This research has shown that both *Xm*LBU-4 and *Xm*LBU-8 produced high gum yields from sucrose, palm sugar, and cassava starch (yields more than 67%) in comparison to the yields from glucose, lactose, malto-dextrin, and cane-molase (yields less than 48%), as seen in Table 3. Among the sugars studied, sucrose in the presence of *Xm*LBU-4 gave the highest yield of xanthan gum; this was the reason why we chose this carbon source and *Xm*LBU-4 for further experiments.

Table 3.
Xanthan gum yield from sugar/starch of *Xm*LBU-4 and *Xm*LBU-8.

Strain	Xanthan gum yield from sugar/starch						
	Glucose	Lactose	Sucrose	Malto-dextrin	Palm sugar	Cassava starch	Cane molasses
*Xm*LBU-4	45.0%	0	76.0%	38.0%	69.3%	67.0%	45.0%
*Xm*LBU-8	47.7%	0	74.3%	30.9%	72.7%	72.3%	41.0%

Previous studies have explored various strategies to optimize xanthan gum fermentation and reduce production costs by using alternative carbon sources. The effects of different carbon sources, including monosaccharides (glucose, fructose, xylose, arabinose, and galactose), disaccharides (sucrose, maltose, and lactose), polysaccharides (soluble starch and potato starch), and polyol sugars (inositol and sorbitol), at a concentration of 2% have been investigated for their impact on xanthan gum production by *X. campestris* [28]. The highest xanthan gum yields were obtained using glucose (14.7 g/l), sucrose (13.2 g/l), maltose (12.3 g/l), and starch (9.7 g/l). In contrast, lactose and polyol sugars such as inositol and sorbitol were found to be unsuitable carbon sources for xanthan gum synthesis.

Other reports indicated that xanthan gum production was lower when using 4% glucose after 72 hours of fermentation with *X. axonopodis* pv. *begoniae* (7.74 g/l), *X. arboricola* pv. *juglandis* (8.22 g/l), the control strain *X. campestris* NRRL B-1459 (7.46 g/l), and *X. axonopodis* pv. *vesicatoria* (6.40 g/l) [11]. In addition, higher gum productions have been reported in a range of 35–70 g/l on laboratory scales [3, 9, 29-31].

The absence of gum production in lactose-containing media in our study may be attributed to the low affinity of bacterial β-galactosidases for lactose hydrolysis [3, 32]. However, lactose utilization appears to be strain-dependent and may also be

influenced by substrate pretreatment. For instance, *X. campestris* strain PTCC1473 produced 16.4 g/l gum from 65 g/l pretreated cheese whey (containing 39 g/l lactose), whereas *X. pelargonii* strain PTCC1474 produced 12.8 g/l gum from 80 g/l pretreated cheese whey (containing 48 g/l lactose) [33].

Cassava starch has also been studied as a carbon source for xanthan gum production. A strain of *X. campestris* (Xcc 8004) produced 17.0 g/l xanthan gum from gelatinized cassava starch but yielded a lower amount (14.3 g/l) when using raw cassava starch [34].

In our study, we demonstrated that palm sugar is a promising carbon source for xanthan gum synthesis. To the best of our knowledge, the use of palm sugar for xanthan gum production has not been previously reported.

Given that our experiments used a sucrose concentration of 30 g/l, we believe that further optimization of sugar concentration and fermentation parameters could enhance the gum productivity of XmLBU-4.

5.3. Xanthan Gum Properties

According to API Specification 13A, xanthan gum powder cultured from Xanthomonas on pure media must be free from other polysaccharides, such as guar gum and starch, as well as other natural polymers or their derivatives. The specification also requires that the granule size be no smaller than 425 microns (mesh size 40) to enhance solubility in water. Additionally, the proportion of granules smaller than 75 microns (mesh size 200) must be limited to prevent dust formation during transfer operations to the hopper [26]. Beyond its role in increasing viscosity, xanthan gum must also be capable of generating a high-viscosity fluid under low flow rate conditions. This property is essential for improving the yield point of drilling fluids, ensuring better suspension and carrying capacity. The xanthan gum produced in this study demonstrates promising results and complies with API Spec. 13A requirements, and proves its suitability for use as a drilling fluid additive.

5.4. The Economic Perspective of Xanthan Gum Production Using Local Resources

Indonesia is currently importing 100% xanthan gum for local use. Most xanthan gum is imported from China, Austria, and the United States [35]. As the second-largest country in the world having rain rainforest area [36], Indonesia holds high potential microbial resources to provide superior xanthan gum-producing bacteria. Extraordinary efforts are needed to search for the best bacterial strain that suits the local substrate availability for commercially feasible xanthan gum production.

Carbohydrate as a carbon source is an indispensable substrate for the synthesis of xanthan gum molecules and substantially contributes to the overall xanthan gum production cost [33]. Utilizing cheaper and more abundant carbohydrates can improve the economy of xanthan gum production. Therefore, low-cost and readily available substrates such as sugar-containing agricultural waste can substitute pure sucrose for industrial-scale xanthan gum production and, at the same time, secure food and feedstock. Data from Figure 4 proved that *X. melonis* LBU-4 can produce gum in a medium with cane molasses as a carbon source; this substrate is a waste from cane sugar production and is often found in Indonesia. Cane molasses has about 77% dry weight, with sucrose content varying from 39.2 to 67.3% of dry matter; it also contains a variety of amino acids [37]. Further experiments are needed to optimize cane molasses concentration to improve xanthan gum production. Some necessary treatments might be needed to reduce inhibitors in cane molasses that can suppress bacterial growth and xanthan gum formation.

6. Conclusion

This study reveals a future potential for xanthan gum production using X. melonis LBU-4, a bacterial strain isolated from local plant sources. Sucrose proved to be the most effective carbon source for xanthan gum fermentation, yielding 22.8 g/l of xanthan gum with high viscosity. Fourier-transform infrared (FTIR) spectroscopy confirmed its structural similarity to commercial xanthan gum, ensuring industrial applicability. The xanthan gum powder produced complies with the American Petroleum Institute (API) 13A standards, demonstrating its potential for use in oil drilling fluids. Our results provide important data for further research on the development of industrial-scale xanthan gum production using local resources. Future studies should focus on optimizing fermentation conditions and exploring alternative substrates to enhance yield and economic viability.

References

[1] Globe Newswire, "Xanthan Gum market size worth USD 1.2 Billion by 2030 at 5.6% CAGR - Report by Market Research Future (MRFR), Market Research Future (MRFR)," 2025. Retrieved: https://www.globenewswire.com/en/news-release/2022/09/23/2521841/0/en/Xanthan-Gum-Market-Size-Worth-USD-1-2-Billion-by-2030-at-5-6-CAGR-Report-by-Market-Research-Future-MRFR.html. 2025.

[2] S. Chaturvedi, S. Kulshrestha, K. Bhardwaj, and R. Jangir, "A review on properties and applications of xanthan gum," *Microbial Polymers: Applications and Ecological Perspectives*, pp. 87-107, 2021. https://doi.org/10.1007/978-981-16-0045-6_4

[3] S. Rosalam and R. England, "Review of xanthan gum production from unmodified starches by Xanthomonas comprestris sp," *Enzyme and Microbial Technology*, vol. 39, no. 2, pp. 197-207, 2006. https://doi.org/10.1016/j.enzmictec.2005.10.019

[4] P. K. Jha, V. Mahto, and V. Saxena, "Study the effects of xanthan gum and aluminium stearate on the properties of oil-in-water emulsion drilling fluids," *Arabian Journal for Science and Engineering*, vol. 41, pp. 143-153, 2016. https://doi.org/10.1007/s13369-015-1646-5

[5] J. Casas, V. Santos, and F. Garcıa-Ochoa, "Xanthan gum production under several operational conditions: Molecular structure and rheological properties ☆ ," *Enzyme and Microbial Technology*, vol. 26, no. 2-4, pp. 282-291, 2000. https://doi.org/10.1016/S0141-0229(99)00160-X

[6] H. Y. Jang, K. Zhang, B. H. Chon, and H. J. Choi, "Enhanced oil recovery performance and viscosity characteristics of polysaccharide xanthan gum solution," *Journal of Industrial and Engineering Chemistry*, vol. 21, pp. 741-745, 2015. https://doi.org/10.1016/j.jiec.2014.04.005

[7] E. Galindo, G. Salcedo, C. Flores, and M.-E. Ramírez, "Improved shake-flask test for the screening of xanthan-producing microorganisms," *World Journal of Microbiology and Biotechnology*, vol. 9, pp. 122-124, 1993. https://doi.org/10.1007/BF00656533

[8] F. Garcıa-Ochoa, V. Santos, J. Casas, and E. Gómez, "Xanthan gum: Production, recovery, and properties," *Biotechnology Advances*, vol. 18, no. 7, pp. 549-579, 2000. https://doi.org/10.1016/S0734-9750(00)00050-1

[9] P. Souw and A. L. Demain, "Nutritional studies on xanthan production by Xanthomonas campestris NRRL B1459," *Applied and Environmental Microbiology*, vol. 37, no. 6, pp. 1186-1192, 1979. https://doi.org/10.1128/aem.37.6.1186-1192.1979

[10] I. M. Bhat, S. M. Wani, S. A. Mir, and F. Masoodi, "Advances in xanthan gum production, modifications and its applications," *Biocatalysis and Agricultural Biotechnology*, vol. 42, p. 102328, 2022. https://doi.org/10.1016/j.bcab.2022.102328

[11] T. Gumus, A. Sukru Demirci, M. Mirik, M. Arici, and Y. Aysan, "Xanthan gum production of Xanthomonas spp. isolated from different plants," *Food Science and Biotechnology*, vol. 19, pp. 201-206, 2010. https://doi.org/10.1007/s10068-010-0027-9

[12] J. G. Vicente, B. Everett, and S. Roberts, "Identification of isolates that cause a leaf spot disease of brassicas as Xanthomonas campestris pv. raphani and pathogenic and genetic comparison with related pathovars," *Phytopathology*, vol. 96, no. 7, pp. 735-745, 2006. https://doi.org/10.1094/PHYTO-96-0735

[13] X. Zhang and M. Babadoost, "Characteristics of Xanthomonas cucurbitae isolates from pumpkins and survival of the bacterium in pumpkin seeds," *Plant Disease*, vol. 102, no. 9, pp. 1779-1784, 2018. https://doi.org/10.1094/PDIS-08-17-1216-RE

[14] G. S. Saddler and J. F. Bradbury, "Xanthomonas," *Bergey's Manual of Systematics of Archaea and Bacteria*, pp. 1-53, 2015. https://doi.org/10.1002/9781118960608.gbm01239

[15] M. H. A. Elella *et al.*, "Xanthan gum-derived materials for applications in environment and eco-friendly materials: A review," *Journal of Environmental Chemical Engineering*, vol. 9, no. 1, p. 104702, 2021. https://doi.org/10.1016/j.jece.2020.104702

[16] L. C. C. da Silva, B. N. Targino, M. M. Furtado, M. A. de Oliveira Pinto, M. P. Rodarte, and H. M. Hungaro, *Xanthan: Biotechnological production and applications. In Microbial production of food ingredients and additives*. Academic Press. https://doi.org/10.1016/B978-0-12-811520-6.00013-1, 2017.

[17] A. Sharma, S. Gautam, and S. Wadhawan, *"Xanthomonas," in Encyclopedia of Food Microbiology (Second Edition), C. A. Batt and M. Lou Tortorello, Eds.* Oxford: Academic Press, 2014.

[18] X. Dai *et al.*, "Construction and application of a Xanthomonas campestris CGMCC 15155 strain that produces white xanthan gum," *Microbiologyopen*, vol. 8, no. 2, p. e00631, 2019. https://doi.org/10.1002/mbo3.631

[19] A. Soltaninejad, M. Jazini, and K. Karimi, "Biorefinery for efficient xanthan gum, ethanol, and biogas production from potato crop residues," *Biomass and Bioenergy*, vol. 158, p. 106354, 2022. https://doi.org/10.1016/j.biombioe.2022.106354

[20] D. D. Saydam, A. Koulouris, and A. C. Dalgıç, "Process simulation of integrated xanthan gum and sorbitol bioproduction: Economic and sensitivity analysis with Taguchi approach," *Process Integration and Optimization for Sustainability*, vol. 4, pp. 279-295, 2020. https://doi.org/10.1007/s41660-020-00117-8

[21] N. Saitou and M. Nei, "The neighbor-joining method: A new method for reconstructing phylogenetic trees," *Molecular Biology and Evolution*, vol. 4, no. 4, pp. 406-425, 1987. https://doi.org/10.1093/oxfordjournals.molbev.a040454

[22] J. Felsenstein, "Confidence limits on phylogenies: An approach using the bootstrap," *Evolution*, vol. 39, no. 4, pp. 783-791, 1985. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

[23] K. Tamura, G. Stecher, and S. Kumar, "MEGA11: Molecular evolutionary genetics analysis version 11," *Molecular Biology and Evolution*, vol. 38, no. 7, pp. 3022-3027, 2021. https://doi.org/10.1093/molbev/msab120

[24] P.-e. Jansson, L. Kenne, and B. Lindberg, "Structure of the extracellular polysaccharide from Xanthomonas campestris," *Carbohydrate Research*, vol. 45, no. 1, pp. 275-282, 1975. https://doi.org/10.1016/S0008-6215(00)85885-1

[25] S. Faria *et al.*, "Characterization of xanthan gum produced from sugar cane broth," *Carbohydrate polymers*, vol. 86, no. 2, pp. 469-476, 2011. https://doi.org/10.1016/j.carbpol.2011.04.063

[26] API, "API specification 13A 18th Edition, August 2010: Specification for Drilling Fluids – Specifications and Testing, 18th ed. American Petroleum Institute," 2010. Retrieved: https://www.api.org/certification-programs/api-monogram-program-and-apiqr/~/media/files/certification/monogram-apiqr/program-updates/13a-18th-edition-purch-guidlines-r1-2011-12-06.pdf. 2010.

[27] K. Simarani, N. I. S. BOKHARI, and H. M. Saleh, "Characterization and identification of Xanthomonas spp. isolated from infected Brassicaceae and selection of potential xanthan gum producer," *Malaysian Applied Biology*, vol. 51, no. 4, pp. 221-227, 2022. https://doi.org/10.55230/mabjournal.v51i4.31

[28] J. Krishna Leela and G. Sharma, "Studies on xanthan production from Xanthomonas campestris," *Bioprocess Engineering*, vol. 23, pp. 687-689, 2000. https://doi.org/10.1007/s004499900054

[29] G. M. Kuppuswami, *Fermentation (Industrial) | Production of Xanthan Gum, in Encyclopedia of Food Microbiology (Second Edition), C. A. Batt and M. Lou Tortorello, Eds*. Oxford: Academic Press, 2014.

[30] S. Kalogiannis, G. Iakovidou, M. Liakopoulou-Kyriakides, D. A. Kyriakidis, and G. N. Skaracis, "Optimization of xanthan gum production by Xanthomonas campestris grown in molasses," *Process Biochemistry*, vol. 39, no. 2, pp. 249-256, 2003. https://doi.org/10.1016/S0032-9592(03)00067-0

[31] M. Abd El-Salam, M. Fadel, and H. Murad, "Bioconversion of sugarcane molasses into xanthan gum," *Journal of biotechnology*, vol. 33, no. 1, pp. 103-106, 1994. https://doi.org/10.1016/0168-1656(94)90103-1

[32] L. Thorne, L. Tansey, and T. J. Pollock, "Direct utilization of lactose in clarified cheese whey for xanthan gum synthesis by Xanthomonas campestris," *Journal of Industrial Microbiology and Biotechnology*, vol. 3, no. 5, pp. 321-328, 1988. https://doi.org/10.1007/BF01569533

[33] S. V. Niknezhad, M. A. Asadollahi, A. Zamani, D. Biria, and M. Doostmohammadi, "Optimization of xanthan gum production using cheese whey and response surface methodology," *Food Science and Biotechnology*, vol. 24, pp. 453-460, 2015. https://doi.org/10.1007/s10068-015-0060-9

[34] Q. Li, W. Yan, K. Yang, Y. Wen, and J.-l. Tang, "Xanthan gum production by Xanthomonas campestris pv. campestris 8004 using cassava starch as carbon source," *African Journal of Biotechnology*, vol. 11, no. 73, pp. 13809-13813, 2012. https://doi.org/10.5897/AJB11.3774

[35] Volza, "Indonesia Xanthan gum imports. Grow-Global," 2024. Retrieved: https://www.volza.com/p/xanthan-gum/import/import-in-indonesia/. 2024.
[36] D. F. R. Cleary and L. Devantier, "Indonesia: Threats to the country's biodiversity," *Encyclopedia of Environmental Health*, vol. 1, pp. 187-197, 2011. https://doi.org/10.1016/B978-0-444-52272-6.00504-3
[37] A. Palmonari *et al.*, "Characterization of molasses chemical composition," *Journal of Dairy Science*, vol. 103, no. 7, pp. 6244-6249, 2020. https://doi.org/10.3168/jds.2019-1764