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Production and characterization of xanthan gum by bacterial isolates

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Abstract:
Xanthan gum is a microbial polymer synthesised by a plant pathogen of *the Xanthomonas* genus. Due to its rheological characteristics and water solubility, it is of enormous commercial significance and has been utilized as a thickening and stabilizing agent in a variety of industries. In this work, the potential for synthesizing xanthan gum in Xanthomonas species isolated from black rot spotted tomatoes, peppers, mango, and bananas was investigated. After washing the leaves in saline solution, a tenfold dilution was made, and aliquots (1 ml) were placed on a nutrient agar plate and incubated for 48 h at 25 °C. Gram staining was made on colonies that appeared yellow. An emulsification test was carried out on bacteria that were gram-negative rods. Potential xanthan gum producers include isolates displaying yellow colonies, gram-negative rods, and stable emulsions on carbon-enriched media. These requirements were satisfied by eight (61.5 %) of the isolates tested. Biochemical analysis of the isolates indicated that they were Xanthomonas species, and they were coded appropriately (BX2, BX3, PX4, MX6, PX7, MX8, TM9, TX11). The molecular analysis of the best two isolates (TM9 and BX3) revealed that they were *Xanthomonas campestris* and *Stenotrophomonas maltophilia*. After 96 h of incubation, *Xanthomonas campestris* and *Stenotrophomonas maltophilia* were the most effective xanthan gum producers, generating 2.10 g/l and 1.63 g/l of xanthan gum, respectively. The apparent viscosity (AV), emulsification index (IE24), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC) were used to characterize the xanthan gums produced. The findings indicated little or no differences between commercially synthesized xanthan gum and produced xanthan gum. However, xanthan gum from *Stenotrophomonas maltophilia* has a higher apparent viscosity (660.6 mPas) that is above those of *Xanthomonas campestris* (526.1 mPas) and commercial xanthan gum (411.3 mPas), respectively. The gums showed structural similarities and exhibited good thermal stability. These findings indicate that Xanthomonas species are viable options for xanthan gum production.

Keywords*:* Plant, *Xanthomonas*, characterization, synthesis, xanthan gum.

I. INTRODUCTION

Several phytopathogenic species in the genus Xanthomonas cause a variety of globally economically significant diseases in monocotyledonous and dicotyledonous crops. Adhesins, extracellular degradative enzymes, lipopolysaccharides, and exopolysaccharides (EPSs) are all produced by Xanthomonas species [1]. *Xanthomonas campestris* is a plant pathogen, an aerobic bacterium that is able to grow both in a complex and a defined medium, it is usually used to produce xanthan gum [2]. The species *Xanthomonas campestris* has a diverse host range from which it can be isolated (mostly plants belonging to the family Brassicaceae) such as cauliflower, spinach, cabbage, rutabaga, and turnip [3].

Xanthan gum is an extracellular complex polysaccharide synthesized by the bacterium *Xanthomonas campestris* [4]. Xanthan gum is made up of repeating pentasaccharide units that contain two molecular structures of glucose, mannose, and one unit of glucuronic acid [5]. Because of its good rheological qualities, pseudo-plasticity, thickening property, and resistance to heat, acid, and alkali, it is frequently employed in foods, cosmetics, medicines, and the oil industry [6]. Xanthan gum is widely utilized in the oil sector, often to thicken drilling mud. Its distinctive high viscosity in low shear can aid in lowering the concentration of suspended particles in drilling fluid [7]. These fluids are responsible for transporting particles cut by the drilling bit back to the surface. Xanthan gum has excellent "low-end" rheology. Xanthan gum is employed in the rheological regulation of tertiary oil recovery and can assist enhance the recovery factor due to its salt resistance and high-temperature resistance. In advanced nations, around 30% to 40% of xanthan gum is employed in the drilling mud and tertiary oil recovery [7].

The application of xanthan gum is expanding in a variety of applications, with an expected annual growth rate of 5-10% [8]-[9]. It was estimated in 2012 by the Fufeng group, one of the largest manufacturers of xanthan gum that 59,000 metric tons of xanthan gum approximately were produced annually. Commercial xanthan gum is now synthesized through

fermentation using glucose and sucrose as carbon sources. Because glucose and sucrose are quite expensive, xanthan gum produced has a high cost [10]-[12].

The high cost of substrate for xanthan gum production has led to finding alternative low-cost and easily accessible fermentation substrates and factors that could favour optimum production of the gum. For the synthesis of xanthan, a variety of low-cost ingredients have been used in place of glucose and sucrose, such as leftover sugar beet pulp, apple juice remnants, chestnut extract, cheese whey, the bark of cocoa residue, date extract, cassava whey, sugar beet molasses, and sugar cane juice among others [13]-[16], [50]. These alternatives can lower the overall cost of xanthan gum manufacturing and add value to the recyclable waste produced by agro-industrial activities, the majority of which are improperly disposed of and have a negative impact on the environment.

Xanthan gum is the most commercially acceptable Food and Drug Administration (FDA) approved microbial polysaccharide, with a global market value of USD 987.7 million predicted by 2020 [17]. In this circumstance, it is essential to discover local Xanthomonas isolates capable of producing xanthan gum. The aim of this study was to screen *Xanthomonas* isolates for xanthan gum synthesis and to perform a full characterization of their products.

II. **Materials and Methods**

A. **Sample Collection**

Leaves showing a black spot of tomato (*Solanum Lycopersicum*) and pepper (*Capsicum annum*) were collected from Nani village in Kaffi Local Government Area (LGA), mango (*Mangifera indica*) and banana (*Musa acuminata*) from Chanchaga, while infected rice seedlings (*Oryza sativa*) were collected from Bida, Niger State, Nigeria. The diseased leaf samples were collected by plucking [18], placing them in clean polyethylene bags, and transporting them to the laboratory for bacterial isolation.

B. **Isolation and Selection of *Xanthomonas* species**

Isolation of *Xanthomonas* species was done using the technique described by Singh, [19]. One gram (1 g) of each of the diseased plant leaf samples revealing a black rot spot was soaked in deionized water for 15 minutes before transferring 1 ml of the water into a test tube containing 9 ml of sterile distilled water for a ten-fold serial dilution. This procedure was carried out five times to obtain a dilution factor of 10^{-6}. 1 ml of dilution 10^{-5} and 10^{-6} each of the samples was withdrawn and plated in duplicates on Nutrient agar (NA) using the pour plate technique and incubated at 25 ºC for 48 h [19],[51].

For the selection of isolates, the incubated plates were observed for characteristic yellow mucoid colonies after 48 h which were then sub cultured repeatedly on NA to obtain pure isolates. The pure isolates were gram-stained and observed under the microscope for isolates showing pink colouration and were rodlike in shape. These were the presumptive colonies of *Xanthomonas* species that were preserved on slants for further characterization. For a further selection of the *Xanthomonas* species, the isolates were screened for their potential to emulsify hydrocarbon; this was done using a xanthan gum production medium of [20] with the following composition: 4 g D-Glucose, 0.6 g yeast extract, 0.4 g K_2HP0_4, 0.01 g $MgS0_4.7H_20$, 200 ml distilled water. 9 ml of the production medium was dispensed into test tubes inundated with 1 ml of crude oil (Bonny light crude, BLC) and sterilized by autoclaving at 121 ºC for 15 minutes. A 24 h culture of the isolates in buffered peptone water was introduced into the medium in test tubes and incubated at 37 ºC for 24 h. Different types of emulsion formed by the organisms were grouped into stable emulsion (oil transformed remained in the emulsified form for 2 h), less stable emulsion (oil was separated out and make a layer on top of the culture broth), unstable emulsion (oil and medium were separated immediately) [21].

C. **Characterization and Identification of Isolates**

The isolates were characterized using standard procedures. Biochemical tests included motility, KOH solubility test, methyl red, Voges Proskauer, H_2S production, TSI test and gram staining, and production of catalase, oxidase, and indole [22]. The isolates were identified by contrasting the isolates' features with those of recognized taxa. The two isolates with considerably high ability in producing xanthan gum were further confirmed using molecular techniques involving amplification and sequencing of the 16s RNA gene [23], DNA extraction, polymerase chain reaction (PCR), gel electrophoresis, purification of modified product [24] and sequencing of DNA fragment.

D. **Xanthan Gum Production**

1). **Inoculum preparation**

The isolates were sub cultured on YDCC (yeast dextrose calcium carbonate agar). (10 g Yeast extract, 20 g Calcium carbonate, 20 g Dextrose, 20 g Agar, 1000 ml Distilled water). The YDCC agar slants were prepared and incubated in an incubator at 37 ˚C for 24 h to verify if there was contamination. The uncontaminated slants were then cultured with the isolates and incubated in an incubator at 30 ˚C for 3 days. The agar slants were examined for orange-coloured organism growth after 3 days. The inoculum for the synthesis of xanthan gum was then obtained from this culture. Cells from 72 h YDCC agar slants incubated at 30 °C were transferred to 250 ml Erlenmeyer flasks containing 50 ml YDCC broth (pH 7.0) and incubated at 30 °C for 48 h to prepare the inoculum [25].

2). **Xanthan estimation**

1 ml of the inoculum was poured into 49 ml of production medium (Yeast extract 3.0 g/l, Glucose 20.0 g/l, K_2HPO_4 5.0 g/l, $MgSO_4$ 0.2 g/l, pH 7.2) in 100 ml conical flask. The cultures were incubated at 37˚C for 96 h [25]. By centrifuging 5 ml of broth at 10,000 revolutions per minute (rpm) for 15 minutes, the polymer was extracted from the fermentation medium. The polysaccharide was precipitated out of the pellet by decanting the supernatant and adding 2:3 volumes of isopropyl alcohol while shaking. Centrifugation at 6000 rpm for 15 minutes separated the precipitate. The supernatant was placed in a micro-centrifuge tube that had been pre-weighed, and it was dried for 18 hours at 60 °C. The xanthan concentration of the fermented broth was estimated as the dry weight just after the micro-centrifuge tube was cooled to 30 °C for 1 h. The xanthan gum concentration was calculated as the dry weight of xanthan gum per litre of culture media [25].

3. **Biomass estimation**

The dry mass of rinsed cell was used to measure growth in the medium. 5 ml broth was segregated in a centrifuge at 10,000 rpm for 15 minutes. Following centrifugation, two fractions were formed: lyophilized biomass and supernatant, which contained xanthan gum. The lyophilized biomass was agitated in deionized water followed by re-centrification to recover the biomass. The biomass that had accumulated in the tubes' bottoms was air-dried in an oven at 60 °C for two hours, then its dry mass gravimetrically taken [25].

E. **Characterization of Xanthan Gum Produced**

1). **Determination of apparent viscosity**

The apparent viscosity of fermentation broth was evaluated at room temperature using a Brookfield system viscometer (Anton Paar, DV1, USA) and spindle number 3 at 40 rpm after 72 h of incubation at 12 °C [26].

2) **Determination of emulsification index (IE_{24})**

The emulsification index (IE_{24}) was calculated using five different oils (Jatropha oil, Castor oil, crude oil, diesel, and vegetable oil). Each sample of oil was poured into tubes in a ratio of 2:3 (v/v) together with the gum suspension (0.1 % w/v), and the mixture was mixed thoroughly for 2 minutes and allowed to rest for 24 h. The height of the homogenized layer and the overall height of the liquid layer were determined [27]. The following formula was used to determine the IE_{24}:

$$IE_{24} = \frac{\text{height of the homogenized layer}}{\text{Overall height}} \times 100$$

3) **Scanning Electron Microscopy (SEM)**

Structural analysis of the xanthan gum produced by the strains was carried out using a SEM (Tescan, Mod. Vega-3 LMU). The gum samples were taped to metallic support containing a sided carbon tape and then coated using a thin coating of gold before being magnified 1000 times using a 3-kV excitation voltage [28].

4) **Fourier Transform Infrared Spectroscopy (FTIR)**

The functional groups of xanthan gum were identified using FTIR analysis. One milligram of xanthan gum was mashed alongside 100 mg of potassium bromide (KBr) and compressed for 30 seconds at 7,500 kg of pressure using a hand presser that is silver-coated to produce transparent granules. The collected granules were put in a Fourier Transform Infrared Spectrophotometer (FTIR-8400S, Shimadzu, Japan), and infrared spectra were recorded between 600 and 4000 waves cm^{-1} with a 32-sample resolution of 4 waves cm^{-1} and a scanning speed detector of 10 kHz [29].

5) **Thermal Gravimetric Analysis (TGA/DTA)**

The xanthan gum was thermogravimetrically evaluated (TGA-Perkin Elmer Model Pyris 1 TGA). Temperature changes ranging from 25 to 70 °C at a rate of heating of 10 °C min^{-1} and an N_2 atmosphere at a flow rate of 20 mL min^{-1} were applied to about 10.0 mg of each sample's original mass. The acquired findings were compared to commercially available xanthan gum [30].

6) **Differential Scanning Calorimetry (DSC)**

The DSC curves were obtained at temperatures ranging from 25 to 400 °C, with a heating rate of 10 °C min^{-1} and a flow rate of 50 mL min^{-1} in a synthetic air dynamic environment. The sample container was an aluminum crucible, and the analyzed samples weighed between 4.0 and 10.0 mg. The values obtained were compared with those of conventional xanthan gum (Sigma) [31].

III. Results

A. **Preliminary screening for bacterial isolates from leaves of the plant for *Xanthomonas* characteristics**

Thirteen bacterial isolates were obtained from plant leaves (Banana, mango, rice, tomato, pepper) and were screened for *Xanthomonas* characteristics based on pigmentation on Nutrient agar, morphological characteristics through Gram's staining, and emulsification ability. Of the thirteen bacterial isolates, eight isolates appeared pale yellow to orange in colour with a shiny surface appearance that was slightly mucoid when incubated on Nutrient agar plates at 37 ˚C for 48 h having no distinct odour. The eight bacterial isolates appeared rodlike in shape with a pinkish colouration when viewed under the microscope (Gram-negative).

The thirteen bacterial isolates from the leaf of Banana (X_2, X_3, X_{12}), Rice (X_1, X_6, X_{10}), Mango (X_5, X_8, X_{13}), Tomato (X_9, X_{11}) and Pepper (X_4, X_7) were subjected to emulsification test in a carbon enriched medium. The results revealed that eight isolates (61.53 %) caused stable emulsion of the oil medium while two (15.38 %) caused less stable emulsion and three (23.07 %) caused unstable emulsion. These results suggested that eight isolates were potential xanthan gum producers.

B. **Characterization and identification of isolates**

Based on the biochemical characteristics of the bacterial strain, The eight isolates were identified and coded as,

Xanthomonas vasicola (BX_2), *Stenotrophomonas* (formerly in the genus Xanthomonas) *maltophilia* (BX_3) *Xanthomonas perforans* (PX_4), *Xanthomonas citri* (MX_6), *Xanthomonas gardneri* (PX_7), *Xanthomonas axonopodis* (MX_8) *Xanthomonas campestris* (TX_9) and *Xanthomonas vesicatoria* (TX_{11})

Molecular analysis was further used to identify isolates (BX_3 and TX_9) with high xanthan gum production capability. After molecular analysis, the bacterial isolates BX_3 and TX_9 were 99 % identical to the 16S ribosomal RNA genes of *Stenotrophomonas maltophilia* and *Xanthomonas campestris*, respectively.

C. **Production of xanthan gum by bacterial isolates**

Eight isolates which caused stable emulsion of hydrocarbon medium were subjected to xanthan gum production. The results revealed that the yield of xanthan gum ranged from 0.02 g/ l to 2.10 g/l (Table 1) after 96 h. Isolate TX_9 showed a considerably higher ability in producing the xanthan gum than the rest of the isolates. This was followed by BX_3 with 1.63 g/L over the same period (Table 1).

D. **Characterization of produced xanthan gum**

1. **Apparent viscosity (AV) of the xanthan gum**

Viscosity measurement for produced xanthan gum from *Xanthomonas campestris*, *Stenotrophomonas maltophilia* and commercial xanthan gum revealed that at a constant revolution

per minute (RPM) of 12 and constant temperature of 40 °C, the AV of xanthan gum (XG) produced by *Xanthomonas campestris* ranged from 526.1 to 597.4 mPa.S at concentrations of 26-49 % while the AV of XG by *Stenotrophomonas maltophilia* ranged from 617.7 to 660.6 mP.aS at a concentration of 26.4-44.3 % (Table 2). Commercial XG had AV of 408.3-411.3 mPa.S at concentration of 16.5-17.2 %.

2. **Emulsification index (EI $_{24}$) of xanthan gum**

Table 3 shows the findings of the emulsification capability of xanthan gum in various oils after 24 h (diesel, crude oil, castor oil, jatropha oil, and vegetable oil). The results revealed that castor oil supported more emulsification (36.45-57.5 %) by xanthan gum produced by the two bacteria. The commercial xanthan gum caused 63.75 % emulsification of castor oil. This was followed by vegetable oil (33.35-47.5 %), *Jatropha* oil (10-37.5 %), crude oil (6.5-17.5 %) and diesel (2.5-8.25 %) in that order (Table 3).

3. **Scanning Electron Microscopy (SEM) of xanthan gum**

At a magnification of 1000, scanning electron microscopy (SEM) was used to study the morphology and surface features of xanthan gum synthesized by *Xanthomonas campestris*, *Stenotrophomonas maltophilia*, and a commercial xanthan gum. The results revealed commercial xanthan gum to present a rough and a more compact structural surface, xanthan gum from *Xanthomonas campestris* had a more disperse heterogenous whitish structure with less surface porosity while xanthan gum produced by *Stenotrophomonas maltophilia* presented a flat whitish surface with a pronounced fiber like structure (Figure 1). For xanthan gum commercial (control), the abundant elements detected were potassium, carbon, phosphorus and iodine with a weight concentration in grams of 28.13, 17.03, 14.55, and 10.95 respectively. Potassium, carbon, calcium and phosphorus with a weight concentration in grams of 22.55, 21.81, 17.99 and 15.88 respectively were also found to be abundant in xanthan gum from *Xanthomonas campestris* while xanthan gum from *Stenotrophomonas maltophilia* had calcium, carbon, zirconium, phosphorus and potassium as abundant elements with atomic weight concentration in grams of 28.95, 15.43, 13.07, 11.95 and 10.92 respectively.

4. **Fourier Transform Infrared Spectroscopy (FTIR) of xanthan gum produced**

The chemical nature of the produced xanthan gum by *Xanthomonas campestris* and *Stenotrophomonas maltophilia* were elucidated using FTIR. The produced spectra for the xanthan gum from FTIR analysis are seen in Figures 2 and 3. The results indicate peaks of various shapes (weak, bending, strong, broad, stretching, asymmetric), each indicating particular functional groups found on the molecular chain of the xanthan gums investigated. Similarly, the infrared absorption pattern revealed the presence of carboxyl, carbonyl, hydroxyl and acetyl groups (Fig. 2 and 3).

5. **Thermogravimetric analysis (TGA/ dTGA)**

The xanthan gum produced was also subjected to thermal gravimetric analysis/ derivative of thermal gravimetric analysis (TGA/dTGA). This analysis provides useful information for a heated substance, showing the degradation initial temperature (T onset), decomposition maximum temperature (T max), and weight loss (W l^3). It was observed that all three samples had two (2) thermal events each, Figure 4, 5 and 6 show the thermogravimetric (TG) and derivative thermogravimetric (dTA) profiles of produced xanthan gum by *Xanthomonas campestris*, *Stenotrophomonas maltophilia* and commercial xanthan gum (control) respectively. *Xanthomonas campestris* CPBF 211 had Tmax of 108.05℃ and 401.00℃ with mass loss of 99.88 % and 95.68 % respectively. *Stenotrophomonas maltophilia* had Tmax of 200.36℃ and 515.61 °C with mass loss (WI^3) of 100.5 % and 66.10 % respectively (Table 6). Commercial xanthan gum (control) had Tmax of 120.88 °C and 411.92 °C with weight loss of 99.96 % and 90.09 % respectively (Table 4). The results revealed that xanthan gum produced by *Stenotrophomonas maltophilia* had higher weight loss than the xanthan gum synthesized by *Xanthomonas campestris* and xanthan gum commercial (Table 4)

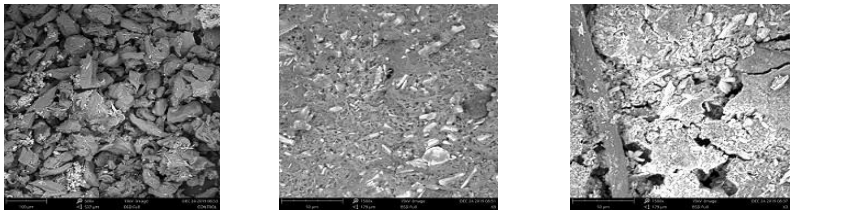
Table 2
Apparent viscosity of xanthan gum

Xanthan gum by:	Apparent viscosity Value (mPa. S)	% concentration of Xanthan gum
Xanthomonas campestris CPBF 211	597.4	23
	564.1	26
	526.1	49
Stenotrophomonas maltophilia IAE127	660.6	26.4
	617.7	36.7
	642.8	44.3
Commercial xanthan gum	408.3	16.5
	408.3	16.8
	411.3	17.2

mPa.S: Millipascal per second.

Table 3
Emulsification index (IE_{24}) of various oils by xanthan gum produced by *Xanthomonas campestris* and *Stenotrophomonas maltophilia*

Xanthan gum by:	Emulsification index (%) Diesel	Crude oil	Castor oil	Jatropha oil	Vegetable oil
Xanthomonas campestris	8.75	17.5	36.45	10	33.75
Stenotrophomonas maltophilia	2.50	6.5	57.5	20	36.25
Commercial xanthan gum (Control)	2.65	10	63.75	37.5	47.5



(a) Xanthan commercial (Control) (b) *Xanthomonas campestris* CPBF 211 (c) *Stenotrophomonas maltophilia* IAE127

Figure 1: Scanning electron micrographs of the produced and commercial xanthan gum

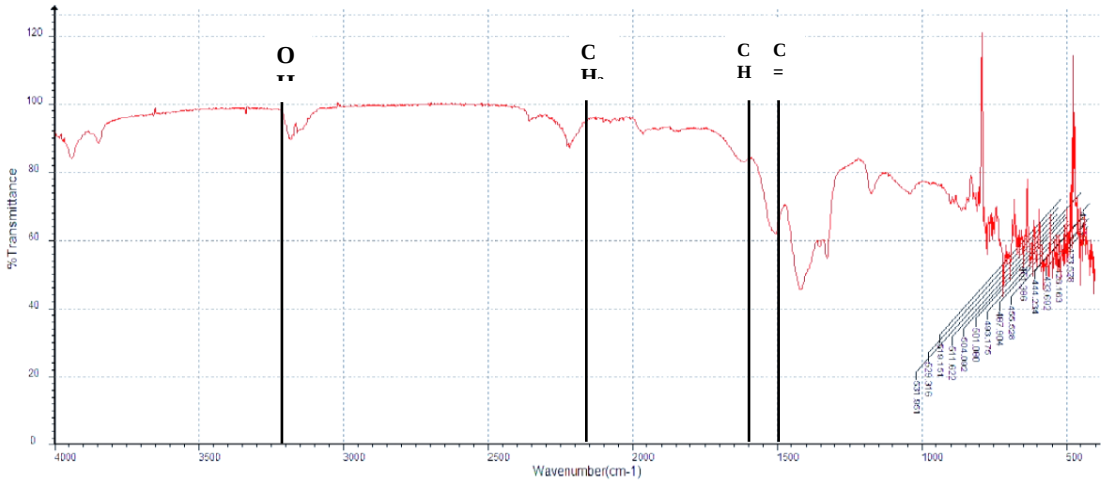


Figure 2: FTIR of xanthan gum synthesized by *Xanthomonas campestris*

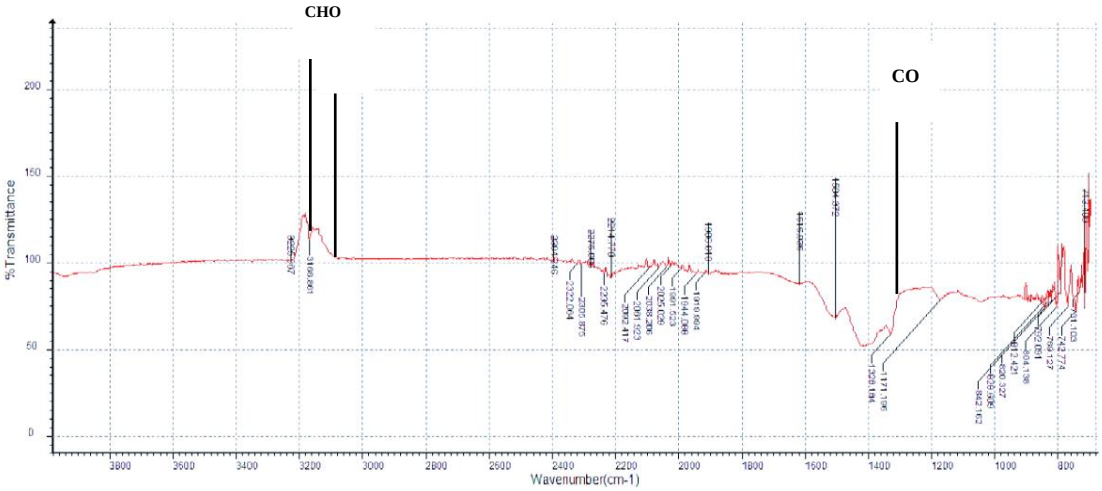


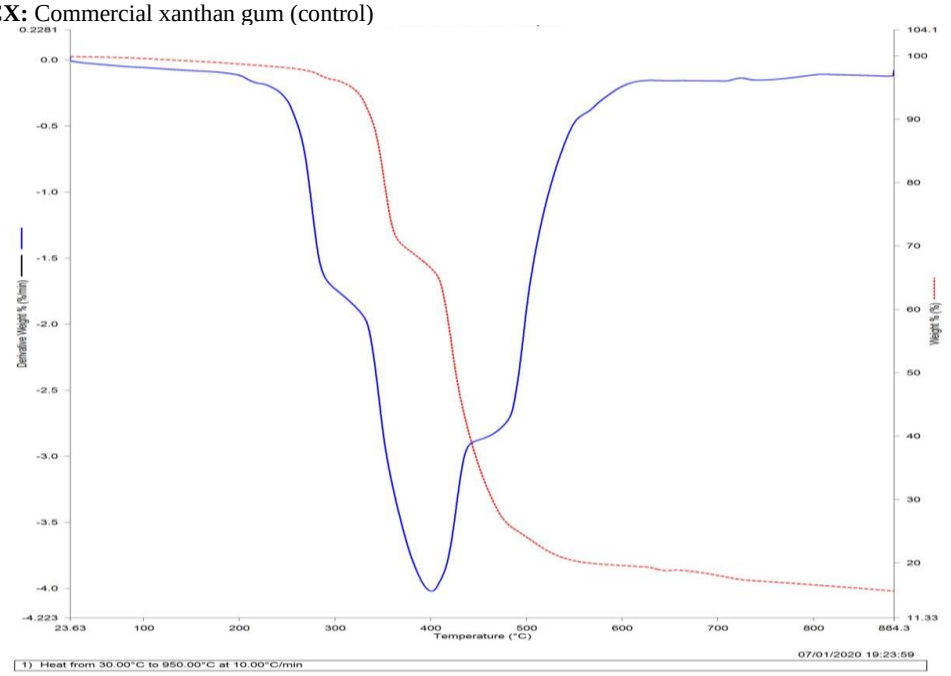
Figure 3: FTIR of xanthan gum synthesized by *Stenotrophomonas maltophilia*

Table 4

Thermogravimetric data (TGA and dTGA) of xanthan gum produced by *Xanthomonas campestris* CPBF 211 and *Stenotrophomonas maltophilia* IAE12

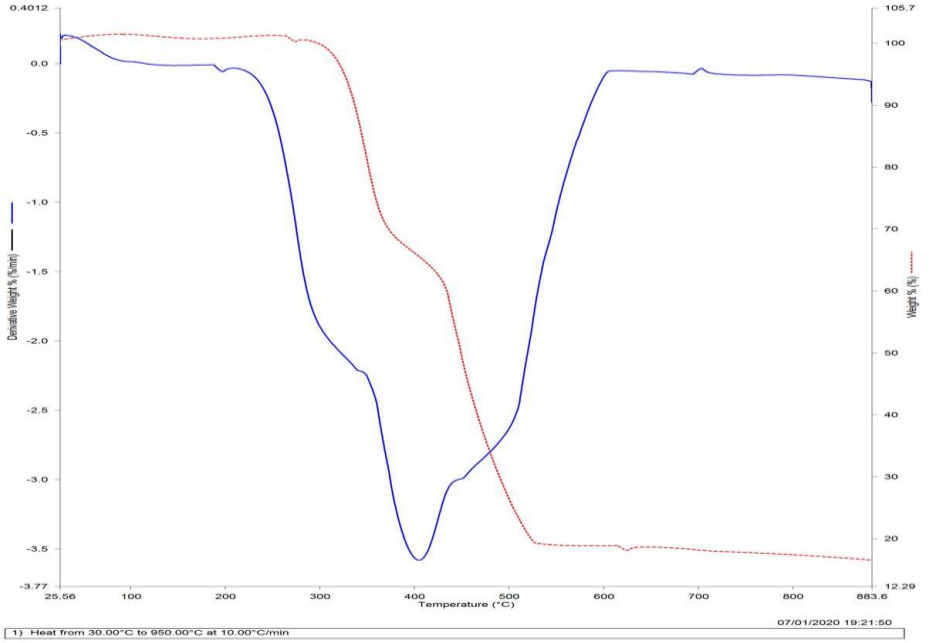
Sample	Events	T_{onset} (˚C)	T_{max} (˚C)	WI^3 (%)
XC	1	23.74	108.05	99.88
	2	309.88	401.00	95.68
SM	1	25.56	200.36	100.5
	2	400.48	515.61	66.10
CX	1	23.19	120.88	99.96
	2	309.84	411.92	90.09

XC: *Xanthomonas campestris***, SM:** *Stenotrophomonas maltophilia* **,**
CX: Commercial xanthan gum (control)



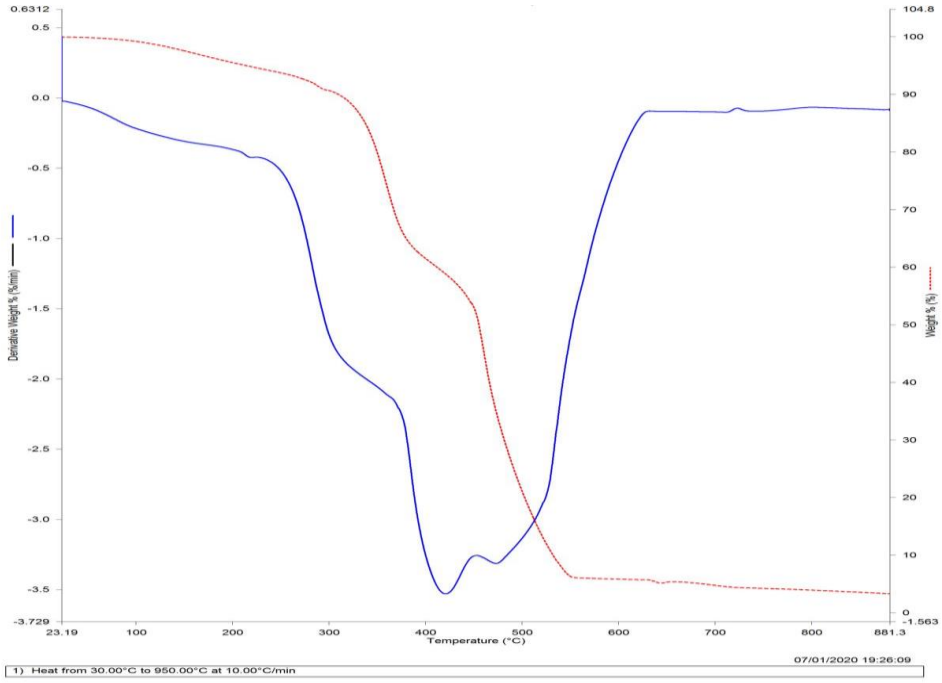
TGA =……. DTA = _____

Figure 4: TGA/ dTGA for *Xanthomonas campestris*



TGA =……. DTA = _____

Figure 5: TGA/ dTGA for *Stenotrophomonas maltophilia*



TGA =……. DTA = _____

Figure 6: TGA/ dTGA for commercial xanthan gum (control)

IV. Discussion

Xanthomonas species were initially selected based on pigmentation on nutrient agar, morphological characteristics and emulsification ability on the carbon source. The organisms exhibited pale yellow to orange pigmentation on nutrient gram-negative time rod and emulsified the carbon source. These qualities helped in the initial selection of X*anthomonas* species. Other investigators [32]-[34], have used these parameters in the primary isolation and selection of *Xanthomonas* species. The yellow/ orange colour of nutrient agar is due to membrane-bounded pigment "Xanthomonas" which may protect bacteria from photobiological damage. The isolated *Xanthomonas* species emulsified hydrocarbon to varying degrees, forming stable, less stable unstable emulsions. This means that the organisms produced surface active agents such as xanthan gum which are useful in various industries including food and petroleum industries [35]-[36].

The *Xanthomonas* species were identified based on the biochemical test as *X. vasicola, X. citri, X. campestris, S. maltophilia, X. axonopodis, X. vesicatoria, X. gardneri* and *X. perforans.* The two best xanthan gum-producing isolates were confirmed by molecular techniques, *Stenotrophomonas maltophilia* (formerly in the genus *Xanthomonas*) and *Xanthomonas campestris*. Other investigators have isolated these organisms from different plant parts where they occur as pathogens [34] [37]-[41].

The viscosity of xanthan gum is essential for its thickening capabilities, as well as its rheological and pseudoplastic effects in aqueous solutions; these attributes are important for industrial applications and are thus the primary indications of xanthan gum quality [11] [42]-[43]. The rheological properties of the produced xanthan gum and the commercial xanthan gum (control) assessed by apparent viscosity (AV) analysis exhibited considerable differences at varying concentrations for both the commercial xanthan gum and the produced xanthan gum in the laboratory at 12 rpm and temperature of 40 ˚C. Generally, the AV of solutions increased with increasing xanthan gum concentration for commercial xanthan gum while the increase in gum concentration for *Xanthomonas campestris* and *Stenotrophomonas maltophilia* led to a decrease in the AV value. The commercial xanthan gum presents a maximum viscosity value of 411.3 followed by that of *Xanthomonas campestris* with a maximum viscosity of 526.1. Maximum viscosity values were obtained from *Stenotrophomonas maltophilia* with 660.6, demonstrating the isolates had a stronger impact on differences in maximum apparent viscosity.

Previous research indicated that various viscosities of xanthan gum had diverse uses; for example, xanthan gum with a high viscosity is commonly used in the petroleum sector, agriculture, and water-based paints [8] [36]. In contrast, low viscosity xanthan gum has a greater dosage and transparency and hence has the potential to be employed in foods and other industries [11] [42]. Because of its low viscosity, xanthan gum has a higher synergistic effect with other additives and gums [42]. Therefore, these gums produced can find application in the food industry as emulsifiers, stabilizers, and suspension agents.

Polysaccharides are introduced to emulsions to enhance their rheological properties, which assists in emulsion stability. Xanthan gum is a typical stabilizer in food emulsions due to it is excellent behaviour as a thickening agent for aqueous solutions. Its capacity to boost emulsion viscosity as well as stability is dependent on the polymer's concentration and structure. The emulsification index results revealed that xanthan gum from both *Xanthomonas campestris* and *Stenotrophomonas maltophilia* gave better IE_{24} with castor oil than other oils reaching values of 67.5 % and 36.45 % respectively. This was followed by vegetable oil with IE_{24} of 33.75-47.5 %, Diesel gave the least emulsification index (2.5 – 8.75 %) which means that the xanthan gum poorly emulsified diesel. The IE_{24} of a conventional xanthan gum mixture (1g L^{-1} w/v) in oil also gave the highest emulsification in castor oil (63.75 %) and the least emulsification with diesel (2.65 %). This implies that castor oil was the best of the five oils used. At a concentration of 1 g L^{-1}, Iyer *et al*. [44] studied polymers synthesize by *Enterobacter cloacae* in cotton, sunflower, coconut, paraffin, jojoba, and peanut oils. The researchers discovered that cotton, coconut, and paraffin oils had an IE_{24} of 60 %, whereas jojoba and sunflower oils had IE $_{24}$s of 65 % and 75 %, respectively. The peak value, however, was from peanut oil (95 %). The IE24 of conventional Arabic and xanthan gum was also examined at about the same concentrations, obtaining readings of 33 % and 61 %, respectively. Exopolysaccharides' (EPS) emulsifying capacity might be linked to functional groups in the biopolymer, such as 6-deoxyhexoses and acetyl moieties, which provide hydrophobicity to the EPS and hence contribute to its emulsifying ability. The presence of a large fraction of hydrophobic amino acids inside the protein moiety further facilitates polysaccharide emulsification [44]. Polymers, such as xanthan gum, have also been widely used to adjust the rheological characteristics of oil-in-water emulsions, hence increasing emulsion stability [45]. Different stress conditions, as well as carbon sources, could disrupt the molecular structure of xanthan gum and result in varying behaviour related to its emulsifying capacity. This is because production process conditions have an impact on various structural properties of xanthan gum, such as molecular weight, acetylation degree and pyruvilation. This has an impact on its physicochemical properties [23].

Scanning electron microscopy revealed xanthan commercial (control) to present a rough and a more compact structural surface, probably due to subjecting the gum to some special treatment such as alkali stress (addition of 2 N NaOH to increase the pH thereby improving the morphology) [46]. The xanthan gum produced by *Xanthomonas campestris* had a more dispersed heterogeneous whitish structure with less surface porosity probably as a result of the sizes of the gum particles and the presence of a white colouration may be due to the high calcium content present in the fermentation medium, Katherine *et al.* [52] also found out a cementitious fibrous smooth amorphous structure with less porosity while studying SEM analysis of xanthan gum synthesis using jack fruit seed extract-based medium. The xanthan gum produced by *Stenotrophomonas maltophilia* presented a flat whitish surface with a pronounced fibre-like structure probably indicating that some of the substrates were still present in the

gum after separation. According to Ahuja *et al*. [28], samples having irregular surfaces impede the passage of particles with irregular shapes and produce blockages.

From the xanthan gum commercial (control) the abundant elements were found to be potassium, carbon, phosphorus and iodine. Potassium, carbon, calcium and phosphorus were also found to be abundant in xanthan gum synthesized by *Xanthomonas campestris* while xanthan gum synthesized by *Stenotrophomonas maltophilia* had calcium, carbon, zirconium, phosphorus and potassium to be abundant elements.

The Fourier Transform-infrared spectrum (FT-IR) is used to detect different functional group present in the xanthan gum produced. For *Xanthomonas campestris* and *Stenotrophomonas maltophilia*, the region investigated includes all spectra bands contained in the window between wave numbers 1000 and 4000 cm^{-1} and 1000 and 3800 cm^{-1}, respectively. The most significant bands recorded in the 4000-1000 cm^{-1} range were: (3200-3350 cm^{-1}: axial deformation of -OH; 2150-2350 cm-1: axial deformation of C-H (could be as a result of absorption of symmetrical and asymmetrical deformation of CH_3 or groups of CH_2) and CHO; 1700-1600 cm^{-1}: axial deformation of C=O carboxylic acid, aldehydes, ester and ketones; 1500-1600 cm^{-1}. The most significant bands reported in the 3800-1000 cm^{-1} range were: (3100-3200 cm^{-1}: axial deformation of C-H and CHO; 1300-1350 cm^{-1}: axial deformation of C O, carboxylic acid, aldehydes, ester and ketones). Gilani *et al*. [29], revealed that commercial xanthan gum contains chemical groups such as: hydroxyl (3386 cm−1), carbonyl (1627 cm−1), carboxyl (1529 cm−1), and acetyl (1160 cm−1).

A comparison of the peak values, representing a specific functional group in *Xanthomonas campestris* and *Stenotrophomonas maltophilia*, revealed that they have almost the same value, According to Faria *et al*. [47] and Li *et al*. [11], the cumulative stretching vibrations of all functional groups closely mimic the structure of xanthan gum.

The thermogravimetric analysis (TGA/DTA) curves of xanthan gum synthesized from *Xanthomonas campestris* and *Stenotrophomonas maltophilia* exhibited two thermal events. The first events is associated to polysaccharides loss of water. Water may be present in the samples because water is rapidly absorbed during the xanthan gum weighing. According to Faria *et al*. [47], the inclusion of Polar groups in xanthan gum's chemical nature allows it to absorb water. The breakdown of the xanthan gum polymer chain was attributed to the second thermal event [27], which began by dissociating groups attached to the side chain, and eventually leading to the unfolding of the main chain. According to Zohuriaan *et al*. [48], the temperature range for commercial xanthan gum degradation was between 251.15 and 330.30 °C, with loss in weight between the range 8.7 and 41.6 %. Xanthan gum synthesized by *Xanthomonas campestris* and *Stenotrophomonas maltophilia* was observed to have a similar thermal stability to the commercial xanthan gum showing their usefulness in procedure that needed extreme temperatures, Faria *et al*. [47], Thermal degradation of xanthan gum from cane juice occurs at temperatures ranging from 220 to 320 ˚C, with a weight loss of 40 % and a maximum loss at 283 ˚C. According to Villetti *et al*. [49], weight loss between 25 and 150 ˚C was connected with the gum's water outlet, whereas carbonization happened between 150 and 400 ˚C and xanthan gum breakdown occurred between 400 and 550 ˚C.

V. Conclusion

Xanthomonas species isolated from diseased plant leaves had the potential to synthesise xanthan gum. *Xanthomonas campestris* and *Stenotrophomonas maltophilia* were discovered to be effective xanthan gum producers, producing 2.10 g/l and 1.63 g/l respectively of the gum after 96 h of incubation. The xanthan gum exhibited good thermal stability. Plant pathogens can produce useful products such as xanthan gum which compares favourably with xanthan gum produced commercially.

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