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Effect of pH, Temperature, Nutrients and incubation time on production of xanthan gum by *Xanthomonas campetris* and S*tenotrophomonas maltophilia*

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ABSTRACT

This study screened *Xanthomonas* species isolated from tomato, pepper, mango and banana with black rot spots for potential to produce xanthan gum. The leaves were washed in normal saline and tenfold dilution was prepared. Aliquots (1ml) were plated on Nutrient agar and incubated at 25 ˚C for 48h. Colonies with yellow pigmentation were gram stained. Gram negative rod bacteria were subjected to emulsification test. Isolates with yellow colonies, gram negative rods and which exhibited stable emulsion in carbon enriched medium were regarded as potential xanthan gum producers. Eight (61.5%) of the isolates screened fulfilled these conditions. Biochemical tests on the isolates revealed that the organisms were *Xanthomonas* species and were coded accordingly (BX_2, BX_3, PX_4, MX_6, PX_7, MX_8, TM_9, TX_{11}). The best two isolates (TM_9 and BX_3) were subjected to molecular analysis and were found to be *Xanthomonas campestris* and *Stenotrophomonas maltophilia*. *Xanthomonas campestris* and *Stenotrophomonas maltophilia* were more efficient xanthan gum producers, yielding 2.10 g/l and 1.63 g/l respectively of xanthan gum after 96 h. The results revealed that as the fermentation time increased, the biomass also increased. The xanthan gum yield by the two organisms was found to increase from 0.92 to 7.6 g/l and 0.99 to 4.55 g/l under optimized conditions of pH: (9.0, 7.0), temperature (25°C) carbon source (02% pineapple peels 0.2% sugarcane bagasse) and nitrogen source (Yeast extract) respectively. The result suggest that *Xanthomonas* species are good candidates for xanthan gum production.

Keywords: *Xanthomonas*, production, optimized condition, xanthan gum, plant.

INTRODUCTION

The genus *Xanthomonas* comprises several phytopathogenic species that cause a variety of worldwide economically important diseases in monocotyledonous and dicotyledonous crops. *Xanthomonas* species produce a range of virulence factors, such as adhesins, extracellular degradative enzymes, lipopolysaccharides, and exopolysaccharides (EPSs) (Bianco *et al*., 2016). *Xanthomonas campestris* is a plant pathogen, an aerobic bacterium that is able to grow both in a complex and a defined medium and usually used to produce xanthan gum (Makut *et al*., 2018). The species *Xanthomonas campestris*has a wide host range from which it can be isolated (mostly plants belonging to the family Brassicaceae) such as cauliflower, spinach, cabbage, rutabaga, turnip (Rodriguez *et al*., 2012).

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Xanthan gum is an extracellular heteropolysaccharide produced by *Xanthomonas* species such as *Xanthomonas campestris* (Habibi and Khosravi-Darani, 2017), Xanthan gum consists of repeated pentasaccharide units which involves two units of glucose, mannose and one unit of glucuronic acid (Darzi *et al*., 2012). It is widely used in foods, cosmetics, pharmaceuticals and oil industries, owing to its excellent rheological properties, pseudoplasticity, thickening property and stability to heat, acid and alkali (Niknezhad *et al*., 2015). In the oil industry, xanthan gum is used in large quantities, usually to thicken drilling mud. Its unique high viscosity in low shear can help lower concentration of the drilling fluid suspended solids (Abidin *et al*., 2012). These fluids serve to carry solids cut by the drilling bit back to the surface. Xanthan gum provides great "low end" rheology. Due to its salt and high temperature resistance, xanthan gum is used in the rheological control of tertiary oil recovery, and can help the recovery factor improve. About 30% to 40% of xanthan gum is used in the drilling mud and tertiary oil recovery in advanced countries (Abidin *et al*., 2012).
The use of xanthan gum is increasing for many applications, and its estimated annual growth is approximately 5–10% (Rosalam and England, 2006; Lopes *et al*., 2015). 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 was produced annually (US International Trade Commission Report, 2013). Currently, commercial xanthan gum is produced by fermentation using glucose and sucrose as carbon sources. Due to the high cost of glucose and sucrose, the produced xanthan gum possesses a high price (Özcan and Öner, 2015; Li *et al.*, 2016; Sharmila *et al.*, 2020).

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 favor optimum production of the gum. Various low-cost materials have been adopted to replace glucose and sucrose for xanthan production which include; waste sugar beet pulp, residue of apple juice, chestnut extract, cheese whey, cocoa bark residue, cassava whey, date extract, cane juice, and sugar beet molasses (Moosavi-nasab *et al*., 2009; Brandão *et al*., 2010; Mabrouk *et al*., 2013; Coasta *et al*., 2014). These alternatives can reduce the total cost of xanthan gum production, as well as add value to the environmentally sustainable waste generated from agro industrial processes since most of these wastes do not receive adequate disposal and cause environmental problems.

In this context, it is important to identify local isolates of *Xanthomonas* that can produce xanthan gum. The aim of this study was to screen *Xanthomonas* isolates for xanthan gum production under various optimized conditions

MATERIALS AND METHODS

Collection of Samples

Leaves showing 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 (Akhtar *et al*., 2008), placed in clean polyethylene bags and transported to the laboratory for the isolation of bacteria.

Isolation and Selection of *Xanthomonas* species

Isolation of *Xanthomonas* species was carried out following the method described by Singh *et al*. (2006). One gram (1g) of each leaf sample from infected plants showing a black rot spot was soaked in sterile distilled water for 15 minutes and a ten-fold serial dilution was done by transferring 1ml of the water into a test tube containing 9 ml of sterile distilled water. This step was repeated five times to obtain a dilution factor of 10^{-6}. 1ml from the dilutions 10^{-5} and $10^{-6}$of each sample was withdrawn and plated in duplicates on Nutrient agar (NA) using the pour plate method and incubated at 25ºC for 48 hours (Singh *et al*. 2006).

For selection of isolates, the incubated plates were observed for characteristic yellow mucoid colonies after 48 h which were then subcultured 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 further selection of the *Xanthomonas* species, the isolates were screened for their potential to emulsify hydrocarbon; this was done using xanthan gum production medium of Chavan and Baig (2016) with the following composition: 4g D-Glucose, 0.6g yeast extract, 0.4g K_2HP0_4, 0.01g $MgS0_4.7H_20$ and 200ml distilled water. Nine millilitres (9ml) of the production medium was dispensed into test tubes inundated with 1ml of crude oil (Bonny light crude, BLC) and sterilized by autoclaving at 121ºC for 15 minutes. A 24 h culture of the isolates in buffered peptone water was introduced into the medium and incubated at 37ºC for 24hours. Different types of emulsion formed by the organisms were grouped into stable emulsion (oil transformed remained in the emulsified form for 2h), 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) (Ijah and Ndana, 2003).

Characterization and Identification of Isolates

The isolates were characterized using the standard procedures. Biochemical tests which included motility, KOH solubility test, methyl red, vogesproskauer, H_2S production, TSI test and gram staining, production of catalase, oxidase and indole (Cheesbrough, 2006). The isolates were identified by comparing their characteristics with those of known 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 (Trindade *et al*., 2007) after DNA extraction, polymerase chain reaction (PCR), gel electrophoresis, purification of modified product (Frank *et al*., 2008) and sequencing of DNA fragment.

Xanthan Gum Production

Inoculum preparation

The media used for subculturing the isolates was yeast dextrose calcium carbonate agar, YDCC. (Yeast extract 10g, Calcium carbonate 20g, Dextrose 20g, Agar 20g, Distilled water 1000ml). The YDCC agar slants were made and incubated at 37˚C for 24 h to check contamination. The slants without contamination were inoculated with the isolates and incubated at 30 ˚C for 3 days. After 3 days, the agar slants were observed for orange colour growth of the organism. This culture was then used as the

inoculum for xanthan gum production. The inoculum was prepared by transferring cells from 72 h YDCC agar slants incubated at 30 °C to 250 ml Erlenmeyer flasks containing 50 ml of YDCC broth (pH 7.0) and incubated at 30 ˚C for 48 h (Kumara *et al*., 2012).

Xanthan estimation

One millilitre of the cultures (inoculum) was transferred to 49ml of production medium (g/l; Glucose 20.0, Yeast extract 3.0, MgSO4 0.2, K_2HPO_4 5.0, pH 7.2) in 100ml Erlenmeyer flask. The cultures were incubated at 37˚C for 96 h (Kumara *et al*., 2012). The polymer was recovered from the fermentation medium by centrifugation of 5ml broth at 10,000 rpm for 15min. The pellet was decanted and the supernatant was precipitated with 2:3 volumes of isopropyl alcohol with shaking to precipitate out the polysaccharide. The precipitate was separated by centrifugation at 6000 rpm for 15min. The residue was transferred to pre-weighed micro-centrifuge tube and dried for 18 h in hot air oven at 60˚C. The micro-centrifuge tube was cooled to 30˚C for 1 h and the dry weight gave the xanthan concentration of the fermented broth. The concentration of xanthan gum was determined as the dry weight of xanthan gum per liter of culture medium (Kumura *et al*., 2012).

Biomass estimation

Growth in the medium was estimated by measuring the dry weight of washed cell mass. Exactly, 5ml broth was separated in a centrifuge at 10,000 rpm for 15 minutes. After centrifugation, two fractions were formed, supernatant containing xanthan gum, and biomass deposited as a pellet. The biomass pellet was resuspended in deionized water for washing and then recentrifuged to reprecipitate the biomass. The biomass deposited at the bottom of the tubes was dried in the oven at 60 °C for 2h and weighed to get the dry mass (Kumura *et al*., 2012).

Effect of Culture Parameters on Biomass and Xanthan Gum Production

Effect of incubation time on biomass and xanthan gum production

The time for biomass and xanthan gum production was determined using three time intervals of 48 h, 96 h and 144 h. The experiments were conducted using conical flasks (250 ml) containing 50 ml xanthan gum production medium. The flasks were sterilized by autoclaving and inoculated with the culture of the organism in each flask. All the flasks were incubated at 30˚C and flasks were withdrawn at different time of incubation keeping all other processes and conditions constant for carrying out surface fermentation (Makut *et al*., 2018). Biomass and xanthan gum were determined (Kumura *et al*., 2012).

Effect of temperature on biomass and xanthan gum production

For the determination of optimum temperature for biomass and xanthan gum production, 50 ml of xanthan gum production medium were sterilized and inoculated with the culture of the organism and incubated at three different temperatures: 25°C, 30°C, 35°C, keeping all other process parameters constant (Makut *et al*., 2018). Biomass and xanthan gum were determined (Kumura *et al*., 2012).

Effect of pH on biomass and xanthan gum production

To determine the optimum pH for biomass and xanthan gum production, three pH levels of 5, 7 and 9 were used. Conical flasks of 250 ml capacity containing 50 ml xanthan gum production medium was adjusted to pH of the medium using 1 N HCL or 1 N NaOH. The flasks were and inoculated with the culture of the organism in each flask. The flasks were incubated at 25 ºC for 96h surface fermentation, keeping other conditions constant. Thus, the effect of different pH levels on production of biomass and xanthan was determined. Biomass and xanthan gum were determined (Kumura *et al*., 2012).

Effect of carbon sources on biomass and xanthan gum production

To study the effect of different carbon sources on biomass and xanthan production, carbon sources (0.2%) such as sugar cane bagasse, pineapple peels and glucose were used in production medium keeping the pH, temperature and time at 7.0, 25 °C and 96 h respectively. Biomass and xanthan gum were determined (Kumura *et al*., 2012).

Effect of Nitrogen sources on biomass and xanthan gum production

To study the effect of different nitrogen sources on biomass and xanthan production, nitrogen sources (0.3%) such as beef extract, ammonium sulphate, peptone, yeast extract were used in production medium keeping the pH, temperature and time at 7.0, 25 °C and 96 h respectively. Biomass and xanthan gum were determined (Kumura *et al*., 2012).

Production of Biomass and Xanthan Gum at Optimized Conditions

In determining biomass and xanthan gum yield by the isolates, the cultural conditions that gave the highest biomass and xanthan gum yield for both *Xanthomonas campestris* and *Stenotrophomonas maltophilia* were used. For *Xanthomonas campestris*, the conditions were temperature 25°C, pH 9, carbon source: pineapple peels 0.3%, Nitrogen source: yeast extract 0.2% while temperature 25°C, pH 5, carbon source: sugarcane bagasse 0.3% and Nitrogen source: yeast extract 0.2% were used for *Stenotrophomonas maltophilia* IAE127. Biomass and xanthan gum were determined (Kumura *et al*., 2012).

Statistical Analysis

Data generated from this study were analyzed using the computer package SPSS (Version 23). Data were expressed as mean ± standard error while two-way analysis of variance (ANOVA) was done to determine significant differences at 5% probability level between xanthan gum and its production conditions. Correlation analysis was also carried out to establish relationship between xanthan gum and the biomass produced at optimal production conditions (Da Silva *et al*., 2018).

RESULTS

Preliminary screening of bacterial isolates from plant leaves 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 at 37˚C for 48h with no distinct odour. The eight

bacterial isolates appeared rodlike in shape with a pinkish coloration 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.

Characterization and identification of isolates

Table 1.1 shows 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}

Table 1.1: Morphology, biochemical characteristics and identification of *Xanthomonas* species

Code	Gram reaction	Shape	Motility	Catalase	KOH solubility	Methyl Red	Starch hydrolysis	Oxidase	H_2S	Indole	TSI	Isolate
TX_9	-	Rod	+	+	+	-	+	-	+	+	+	*Xanthomonas campestris*
BX_3	-	Rod	+	+	+	+	-	-	-	+	+	*Stenotrophomonas maltophilia*
PX_4	-	Rod	+	+	+	-	+	-	+	+	+	*Xanthomonas perforans*
BX_2	-	Rod	+	+	+	-	+	-	+	+	+	*Xanthomonas vasicola*
PX_7	-	Rod	+	+	+	-	+	-	+	+	+	*Xanthomonas gardneri*
MX_8	-	Rod	+	+	+	-	+	-	+	+	+	*Xanthomonas axonopodis*
TX_{11}	-	Rod	+	+	+	-	+	-	+	+	+	*Xanthomonas vesicatoria*
MX_6	-	Rod	+	+	+	-	+	-	+	+	+	*Xanthomonas citri*

(+): Positive, (-): Negative result, TSI: Triple sugar iron agar

The isolates (BX3 and TX9) with efficient xanthan gum production potentials were further identified using molecular analysis. Plate I is the image of agarose gel electrophoresis to PCR amplified DNA of the isolates. The bacterial isolates, BX3 and TX9 were 99% identical to *Stenotrophomonasmaltophilia* and *Xanthomonascampestris* 16S ribosomal RNA gene after molecular characterization respectively.

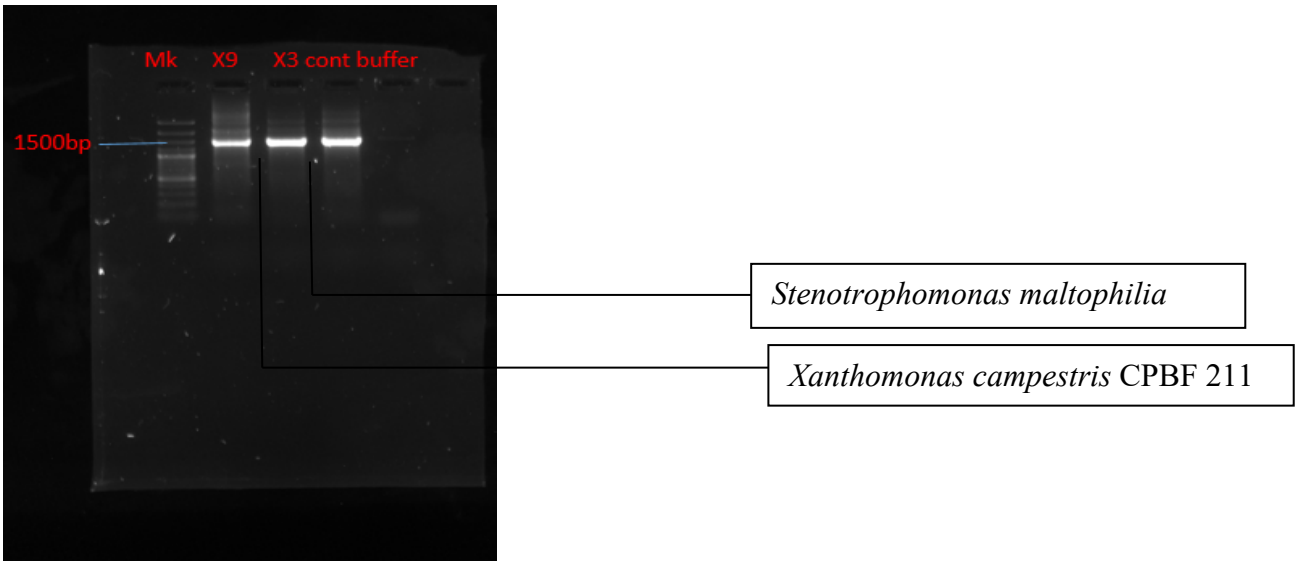

Plate I: Image of Agarose gel electrophoresis to PCR amplified DNA

Key: MK=DNA ladder 1500bp, X9 = sample (*Xanthomonas campestris* CPBF 211), X3 = sample (*Stenotrophomonas maltophilia* IAE127), Buffer, Approximate size of the organism = 1500.

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 2.10g/l (Table 1.2) 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 1.63g/L over the same period (Table 1.2).

Table 1.2: Yield of xanthan gum by *Xanthomonas* species from plant leaves

Isolate	Xanthan gum yield (g/L)
Xanthomonas vasicola BX_2	1.15 ± 0.058
Stenotrophomonas maltophilia BX_3	1.63 ± 0.054
Xanthomonas perforans PX_4	0.95 ± 0.012
Xanthomonas citri MX_6	0.55 ± 0.105
Xanthomonas gardneri PX_7	0.02 ± 0.000
Xanthomonas axonopodis MX_8	1.30 ± 0.050
Xanthomonas campestris TX_9	2.10 ± 0.297
Xanthomonas vesicatoria TX_{11}	1.19 ± 0.040

*Values are presented as mean ± standard error

Effect of Culture Parameters on Biomass and Xanthan Gum Production

Effect of incubation time on biomass and xanthan gum production

Table 1.3 shows the effect of incubation time on biomass and xanthan gum production after 48h, 96h, and 144h of incubation. The results revealed that, as the biomass increased, the xanthan gum production by the two organisms also increased. Maximum xanthan gum (1.36-3.64 g/L) and biomass production (4.06-6.74 g/L) from the organism was obtained after 144h. It was observed that, *X campestris* produced more xanthan gum and biomass than *S. maltophilia*. Xanthan gum produced by the two organisms after 48h was significantly ($p>0.05$) different from the amount produced after 96h and 144h. No significant ($p>0.05$) differences existed among the periods of the production of biomass by the two bacteria (Table 1.3).

Effect of temperature on biomass and xanthan gum production

Table 1.4 shows the effect of temperature on biomass and xanthan gum production at 25ºC, 30ºC and 35ºC. Maximum xanthan gum and biomass production by *Xanthomonas campestris* was at 25ºC and 30ºC respectively while maximum xanthan gum and biomass production by *Stenotrophomonas maltophilia* was at 25ºC and 35ºC respectively (Table 1.4).

Effect of pH on biomass and xanthan gum production

Table 1.5 shows the effect of pH on biomass and xanthan gum production. The results revealed that, as the biomass decreased the xanthan gum produced by *Xanthomonas campestris* increased while the xanthan gum produced by S*tenotrophomonas maltophilia* decreased as the biomass increased (Table 1.5). Maximum xanthan gum (2.37- 4.29 g/l) and biomass production (2.56- 5.25 g/l) by *Xanthomonas campestris* was obtained at pH 9 and pH5 respectively while maximum xanthan gum (3.02- 4.00 g/l) and biomass production (2.37- 4.29 g/l) by S*tenotrophomonas maltophilia* was obtained at pH 5 and pH 7 respectively. It was observed that, *Xanthomonas campestris* produced more biomass than S*tenotrophomonas maltophilia* while S*tenotrophomonas maltophilia* produced more xanthan gum than *Xanthomonas campestris*. Xanthan gum and biomass produced by the two organisms were not significantly different ($P<0.05$) at pH 5-9 (Table 1.5).

Effect of different carbon sources on biomass and xanthan gum production

The production of biomass and xanthan gum was studied using three carbon sources; glucose, pineapple peels and sugarcane bagasse (Fig.1). The results revealed that pineapple peels and sugarcane bagasse supported the highest biomass (6.99 g/l) and xanthan gum (3.84 g/l) production respectively in S*tenotrophomonas maltophilia*. Similarly, sugarcane bagasse supported the highest xanthan gum (4.82 g/l) and pineapple peels gave the highest biomass (3.00 g/l) yield in *Xanthomonas campestris* (Fig.1). It was observed that *Xanthomonas campestris* produced more xanthan gum than S*tenotrophomonas maltophilia*.

Effect of different nitrogen sources on biomass and xanthan gum production

Figure 2 shows the results of the organism cultivated in production medium containing various nitrogen sources such as peptone, yeast extract, and ammonium sulphate. The results revealed that yeast extract supported the highest biomass and xanthan gum production in S*tenotrophomonas*

maltophilia while yeast extract and ammonium sulphate gave the highest xanthan gum and biomass respectively in *Xanthomonas campestris*.

Production of biomass and xanthan gum under optimized culture conditions

Xanthan gum and biomass production under optimal conditions (pH: 9.0, temperature: 25ºC; carbon source: 0.2% pineapple peels, nitrogen source: yeast extract) for *Xanthomonas campestris* and (pH: 7.0, temperature: 25ºC, carbon source: 0.2% sugarcane bagasse, nitrogen source: yeast extract) for *Stenotrophomonas maltophilia* respectively were observed. It was also observed that there was an increase in both biomass and xanthan gum production as the incubation time extended from 24 to 96 hours, maximum yield was observed at 96 hours (Table 1.6). Statistical analysis revealed that there were no significant differences (P> 0.05) in biomass production by the two organisms. However, significant difference (p<0.05) existed in the xanthan gum production by the two organisms, particularly after 72-96 hours (Table 1.6).

Table 1.3: Biomass and xanthan gum produced by bacterial by isolates at various incubation time

	Biomass (g/L)		Xanthan gum (g/L)	
Incubation time (Hours)	**XC**	**SM**	**XC**	**SM**
48	5.91 ± 0.043 [a]	3.83 ± 0.098 [a]	1.24 ± 0.054 [b]	1.20 ± 0.236 [a]
96	6.73 ± 0.574 [a]	4.04 ± 0.450 [a]	3.63 ± 0.012 [a]	1.36 ± 0.063[a]
144	6.74 ± 0.580 [a]	4.06 ± 0.445 [a]	3.64 ± 0.012 [a]	1.36 ± 0.066 [a]

Values are presented as mean ± standard error. Values with the same superscript in the same column are not significantly different at p≥0.05.

XC: *Xanthomonas campestris*, **SM:** *Stenotrophomonas maltophilia*

Table 1.4: Biomass and xanthan produced by bacterial isolates at different temperature levels

Incubation temperature	Biomass (g/L)		Xanthan gum (g/L)	
(˚C)	**XC**	**SM**	**XC**	**SM**
25	4.58 ± 0.040 [a]	3.75 ± 0.387 [a]	6.43 ± 0.395 [a]	3.75 ± 0.387 [a]
30	5.64 ± 0.367 [a]	3.76 ± 0.364 [a]	1.71 ± 0.008 [b]	1.71 ± 0.067 [a]
35	4.20 ± 0.003 [a]	4.76 ± 0.502 [a]	1.56 ± 0.020 [a]	1.54 ± 0.502 [a]

Values are presented as mean ± standard error. Values with the same superscript in the same column are not significantly different at p>0.05.

Table 1.5: Biomass and xanthan gum produced by bacterial isolates at different pH levels

	Biomass (g/L)		Xanthan gum (g/L)	
pH	**XC**	**SM**	**XC**	**SM**
5.0	5.25 ± 0.378 [a]	2.74 ± 0.049 [a]	2.37 ± 0.038 [a]	4.00 ± 0.087 [a]
7.0	3.97 ± 0.072 [a]	3.73 ± 0.115 [a]	3.70 ± 0.170 [a]	3.06 ± 0.081[a]
9.0	2.56 ± 0.032 [a]	3.60 ± 0.087 [a]	4.29 ± 0.430 [a]	3.02 ± 0.026 [a]

Values are presented as mean ± standard error. Values with the same superscript in the same column are not significantly different at p>0.05.

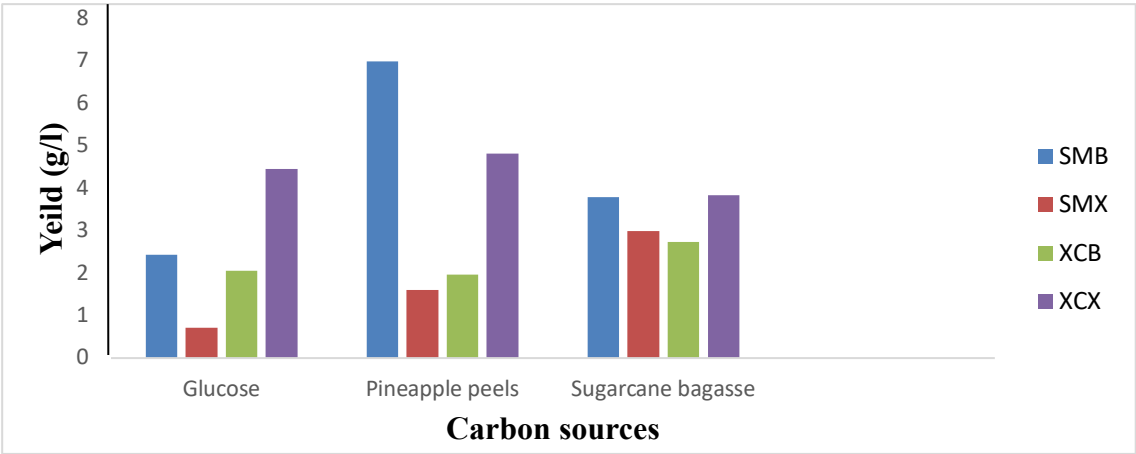

Figure 1: Yield of biomass and xanthan gum by *Xanthomonas campestris* and *Stenotrophomonas maltophilia* on different carbon sources

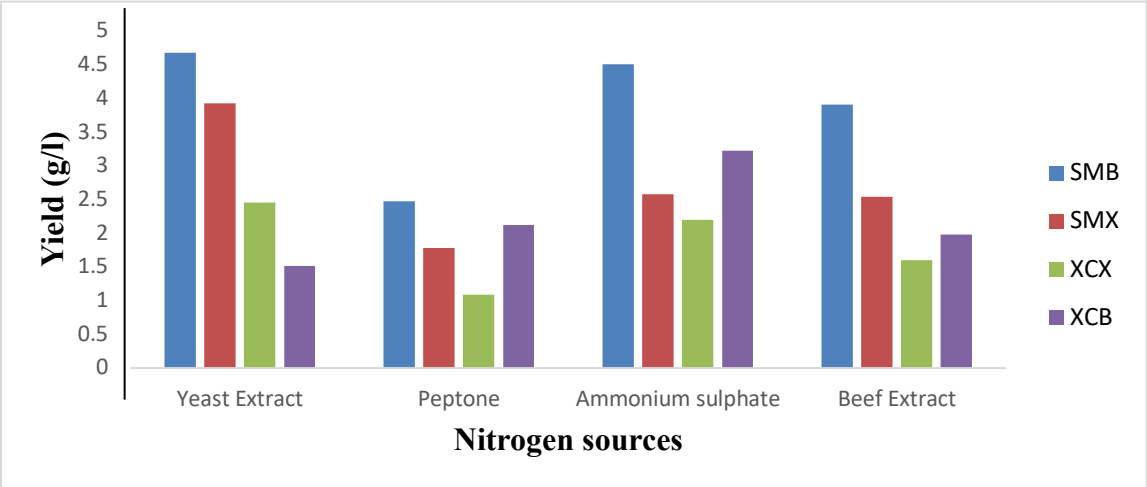

Figure 2: Yield of biomass and xanthan gum by *Xanthomonas campestris* And *Stenotrophomonas maltophilia* on nitrogen sources

Table 1.6: Yield of biomass and xanthan gum under optimized culture conditions

Incubation time	Biomass (g/L)		Xanthan gum (g/L)	
(Hours)	XC	SM	XC	SM
24	0.96 ± 0.021 [a]	2.52 ± 0.028 [a]	0.92 ± 0.113 [a]	0.99 ± 0.014 [a]
48	1.32 ± 0.247 [a]	3.02 ± 0.028 [a]	1.22 ± 0.282 [a]	1.17 ± 0.035 [a]
72	3.69 ± 0.438 [a]	4.05 ± 0.028 [a]	4.40 ± 0.000 [b]	3.39 ± 0.014 [a]
96	6.50 ± 2.828 [a]	6.80 ± 0.056 [a]	7.26 ± 0.197 [b]	4.51 ± 0.014 [a]

Values are presented as mean ± standard error. Values with the same superscript in the same column are not significantly different at $p \geq 0.05$.

DISCUSSION

Xanthomonas species were initially selected based on pigmentation on nutrient agar, morphological characteristics and emulsification ability on carbon source. The organisms exhibited pale yellow to orange pigmentation on nutrient agar, gram negative rod and emulsified the carbon source. These qualities helped in the initial selection of X*anthomonas* species. Other investigators (Roumagnac *et al*., 2004; Ogolla and Neema, 2019; Izadiyan and Taghavi, 2020) have used these parameters in the primary isolation and selection of *Xanthomonas* species. The yellow/ orange colour on nutrient agar is due to a membrane bound pigment "xanthomonadin" which may protect bacteria from photobiological damage (Roumagnac *et al*., 2004). The isolated *Xanthomonas* species emulsified hydrocarbon to varying degrees, forming stable, less stable of 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 (Ijah and Olarinoye, 2012; Paraniraj and Jayaraman, 2011; Habibi and Khogravi- Darani, 2017).

The *Xanthomonas* species were identified based on 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 (Beattie, 2007; Tonu *et al*., 2013; Abbasi and Weselowski, 2015; Sharma, 2018, Newberry *et al.*, 2019; Izadiyan and Taghavi, 2020).

Biomass and xanthan gum production by both *Xanthomonas campestris* and *Stenotrophomonas maltophilia* was found to increase between 48h and 96h, but as incubation time extended from 96h to 144h, there was a little/no increase in biomass as well as the xanthan gum produced by the organisms. This may be due to the presence of acid groups in the biopolymer which resulted to a decrease in pH as the fermentation time extended beyond 96h (Psomas *et al*., 2007; Borges *et al*., 2009; Lopes *et al*., 2015). Hence optimum time of xanthan gum production was 96h, this agrees with the study by Chavan and Baig. (2016) who found a relationship of biomass and xanthan production by *Xanthomonas*

campestris. The investigators found that, there was a decrease in both biomass and xanthan gum produced after 96h of incubation. However, significant differences existed between the amount of xanthan gum produced by *Xanthomonas campestris* at 48 and 96h of incubation at $p<0.05$. Earlier studies have shown that the maximum xanthan production was produced after 48h and increased with increase in time (Mohan and Babitha, 2010: Palaniraj and Jayaraman, 2011).
It was observed that, as temperature increased from 25°C to 35°C, the production of biomass increased and xanthan gum production decreased in *Stenotrophomonas maltophilia*. In *Xanthomonas campestris* increase in temperature from 25°C to 35°C led to a decrease in both biomass and xanthan gum production. This may be due to the fact that the organism is not thermotolerant and therefore, could not withstand slightly higher temperature other than the optimum (30°C ± 2) to give xanthan gum (Lopes *et al*., 2015: Da silver *et* al., 2018: Makut *et al*., 2018). There was a significant difference in the amount of xanthan gum produced by *Xanthomonas campestris* at 25°C and 30°C at $p<0.05$. Therefore, it was clear that the optimum temperature for xanthan gum production in *Stenotrophomonas maltophilia* was 30°C, whereas the optimum temperature for *Xanthomonas campestris* was less than 30°C. Maximum production of biomass and xanthan gum was found to be 4.76 g/L and 1.71 g/L respectively for *Stenotrophomonas maltophilia*, while it was 6.43g/L and 5.64 g/L respectively for *Xanthomonas campestris*. This implies that *Xanthomonas campestris* is a better strain to use for xanthan gum production at these conditions.

The effect of pH on biomass and xanthan gum production revealed that, as pH increased from 5 to 9, biomass produced by *Xanthomonas campestris* decreased while xanthan gum increased. Highest yield of biomass and xanthan gum was obtained at pH 5 (5.25g/l) and pH 9 (4.29 g/l) respectively. This agrees with most authors (Psomas *et al*., 2007; Palaniraj and Jayaraman, 2011; Silva *et al*., 2009; Gumus *et al*., 2010; Lopes *et al*., 2015) who reported that for xanthan gum production, neutral pH was ideal for the growth of *Xanthomonas campestris*. However, pH may decrease during fermentation to around 5.0 due to acid groups present in the biopolymer. Also, it was observed from the study that as the pH increased from 5 to 9 the xanthan gum produced by *Stenotrophomonas maltophilia* decreased while the biomass increased, implying that optimal xanthan gum production was at pH 7. There was no significant difference ($p>0.05$) in the biomass and xanthan gum produced by the two organisms between pH 5 and 9.
Effect of carbon source on biomass and xanthan gum production showed that, *Stenotrophomonas maltophilia* produced the highest biomass (2.43g/L) and xanthan gum in glucose medium and *Xanthomonas campestris* gave the highest xanthan gum yield of 4.46g/L, in sugarcane bagasse containing medium, *Stenotrophomonas maltophilia* produced the highest biomass of 3.79g/L while *Xanthomonas campestris* gave the highest xanthan yield of 3.84g/L. *Stenotrophomonas maltophilia* cells grown in pineapple peels gave the highest biomass yield of 6.99 g/l, while *Xanthomonas campestris* grown in the same medium gave the highest xanthan gum yield of 4.82 g/L among the carbon sources tested. The present results agree with Souw and Demain (1979) and Kawahara and Obata (1998) who reported that, maximum xanthan production was obtained when sucrose was used as a carbon source. The total soluble sugar found in pineapple is predominantly sucrose (Sangprayoon *et al*., 2019). The least xanthan gum yield was found in the medium containing glucose (0.72g/L). Glucose concentrations of less than 2 to 5% are not effective for maximum cell growth. Also, high concentration of glucose has no significant effect on cell growth and xanthan gum production (Amanullah *et al*., 1998; Leela and Sharma, 2000; Niknezhad *et al*., 2015).

The effect of nitrogen sources on biomass and xanthan gum production indicated that biomass was highest in yeast extract (4.68g/L) for *Stenotrophomonas maltophilia* while *Xanthomonas campestris* had highest biomass (2.20g/L) in ammonium sulphate. Both *Stenotrophomonas maltophilia* and *Xanthomonas campestris* had the highest yield of xanthan gum in yeast extract medium (3.93g/L and 3.22g/L) respectively. This implies that the amount of nitrogen required in the fermentation media is less than that required in the growth medium of the organism. Ammonium sulphate therefore is a better substrate for biomass accumulation while xanthan gum yield was higher with nitrate used as nitrogen source (Muniyasamy *et al*., 2019). This is in agreement with the work of Palaniraj and Jayaraman (2011) who reported highest xanthan gum production when yeast extract was used as nitrogen source in the medium.

When the conditions were optimized (pH: 9.0, 7.0; temperature: 25ºC; carbon source: 0.2% pineapple peels, sugarcane bagasse; nitrogen source: yeast extract for *Xanthomonas campestris and Stenotrophomonas maltophilia* respectively) xanthan gum and biomass increased in yield by both *Stenotrophomonas maltophilia* and *Xanthomonas campestris*; maximum yield of xanthan gum and biomass for both organisms was observed at 96 h of incubation. Correlation analysis revealed that there was a strong positive correlation between biomass and xanthan gum produced by the organisms and it was highly significant at $P< 0.01$.

CONCLUSION

Xanthomonas species isolated from diseased plant leaves had the potentials for xanthan gum production. *Xanthomonas campestris* and *Stenotrophomonas maltophilia* were efficient xanthan gum producers, yielding 2.10 g/l and 1.63 g/l respectively of xanthan gum after 96 h. the yield was found to increase under optimized conditions of pH, temperature, carbon and nitrogen sources. There was a strong positive correlation between biomass and xanthan gum produced by the organisms. Plant pathogen can produce useful products such as xanthan gum which compares favourably with xanthan gum produced commercially.

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Declaration

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Availability of data- yes
Code availability- SPSS version 23
Authors contribution-

J.T.kure- performed the research and wrote the paper
U.J..J Ijah- Design and supervise the study
B.T Kure- analyse data
U. Ahmadu- Laboratory Technical assistance
S.B. Salubuyi- contributed new reagents.

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