Cultural and Molecular Characterization of Bacterial Contaminants from Pounded Yam Sold Along Major Roads in Makurdi Metropolis, Benue State, Nigeria

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Abstract: Roadside foods are often contaminated by various pathogenic bacteria, which can cause food-borne diseases. This study was aimed at using molecular methods to identify bacterial contaminants in pounded yam sold along major roads in Makurdi metropolis, Benue State, Nigeria. Isolation and biochemical characterization of the bacterial contaminants in pounded yam samples was done on Nutrient agar, Eosine methylene blue and Mannitol salt agar. The ZR DNA Miniprep was utilized in DNA extraction. BigDye Terminator kit was used for sequencing on a 3510 ABI sequencer. BLAST analysis was performed on the sequences. Results of isolation and biochemical characterization revealed six bacterial genera: *Pseudomonas* spp, *Bacillus* spp, *Staphylococcus* spp, *Escherichia coli*, *Klebsiella* spp and *Proteus* spp. Lowest bacterial occurrence of 11.98 % was recorded in pounded yam samples from Gboko, Wadata samples accounted for the highest percentage occurrence of 17.19 %. *Escherichia coli* and *Staphylococcus* spp were implicated as the most contaminating bacteria accounting for 20.84 and 19.27 % respectively. *Bacillus* spp was the least contaminating bacteria accounting for 8.33 %. The 16SrRNA gene amplification of the isolated bacterial DNA was at 1500 bp. Result of BLAST analysis revealed the bacterial contaminants in the pounded yam samples as *Staphylococcus aureus* strain B3A22 (93.80 % pairwise identity), *Staphylococcus sciuri* strain LZH-22 (93.10 % pairwise identity) and Escherichia coli strain G1F12 (76.70 % pairwise identity). The bacterial contaminants in the pounded yam contain some species involved in food-borne illnesses. Therefore, hygienic way of food preparation is of utmost importance.

Keywords: Molecular characterization, Bacteria, Pounded yam; Food contamination.

INTRODUCTION

The sale of foods such as maize, processed cassava (foo foo), rice, plantain and pounded yam is now a common practice among food vendors in Nigeria (Ameko *et al.,* 2012). Despite the economic and nutritional benefits of roadside foods, their consumption has been implicated as being potentially able to increase the risk of food borne diseases. This is because roadside foods are readily contaminated by different sources resulting in food borne diseases, one of which is travelers' diarrhea. Food poisoning can also occur as a result of entrance of chemical or the ingestion of toxicants (Al Banna *et al.,* 2022).

The safety of roadside foods is always a matter of concern because in most cases, they are prepared and sold under unhygienic conditions by the vendors who in some cases are illiterates and do not practice hygiene (Okojie and Isah, 2014). As such, the chances of contamination of these foods are high. The water used for cleaning and drinking purposes is also prone to contamination due to unhygienic storage and handling and proper garbage removal facilities are in some cases, not available. This in turn would lead to poor environmental conditions, which could heighten the chances of the food contamination.

Identification of the bacterial contaminants in street-vendor-produced pounded yam is necessary to promote food safety practices in food production and consumption, especially in developing nations.

Furthermore, there is very little information on the molecular characterization of bacteria isolated from pounded yam sold along major roads in Makurdi metropolis, Benue State.

Therefore, this study is an attempt to identify the bacterial contaminants of pounded yam using cultural and molecular methods, with a view to raising awareness among consumers about the safety or otherwise of such vended pounded yam.

MATERIALS AND METHODS

Sampling site

A total of seven food vending sites, which served at least thirty (30) customers per day were selected for sampling. They included: Wurukum, Wadata, Lafia road (North Bank), Gboko road (Gyado Villa, Gaadi, Fiidi), Otukpo road (Idye, Kanshio, Apir), Naka road (Ankpa Quarters, Modern Market) and High level.

Sample collection

A total number of 140 samples of pounded yam at 20 samples from each sampling site; were randomly collected in sterile containers with seals and transported to the laboratory of the Department of Microbiology, J. S. Tarka University, Makurdi for microbiological analysis.

Isolation and biochemical identification of bacteria

Twenty five (25) grams of each pounded yam sample were suspended in 225 mL of buffered peptone water and stirred to form a suspension. The colloidal liquid suspension formed the stock sample from which dilutions were made to obtain 10-fold serial dilutions. A 0.1 mL of it was transferred into sterile Nutrient agar using spread plate method and incubated at 37 ^{0}C for 24 hours (Feglo and Sakyi, 2012). After 24 hours of incubation, growth on the inoculated plates was examined for colonies. The colonies were picked and re-inoculated in repeated sub culturing unto fresh Nutrient agar to obtain pure cultures from which gram staining, morphological and biochemical characterization were done (Cheesbrough, 2006). Eosin methylene blue (EMB) and mannitol salt agar (MSA) were used for the confirmation of *E. coli* and *S. aureus* respectively. Three of the bacterial isolates The agarose gel was put in the gel box (electrophoresis apparatus) once it had set.

identified using biochemical characterization was further subjected to molecular identification to confirm the bacterial species present in the pounded yam samples. Pure cultures of the colonies were standardized and 0.1 mL of each was transferred into a sterile Nutrient broth and incubated at 37 ^{0}C for 24 hours (Feglo and Sakyi, 2012).

Amplification of gene of interest

The DNA extraction was carried out according to the protocol of Ojo-Okunola *et al.* (2020). The PCR mixture contained 2 liters of DNA template, 1 liter each of 10 mM forward and reverses primers, 12.5 liters of Taq 2X Master Mix from and 8.5 liters of nuclease-free water.

Initial denaturation was done at 94˚C for 5 minutes, followed by 36 cycles of denaturation at 94˚C for 30 seconds. Annealing was done at 55˚C for 30 seconds and elongation at 72˚C for 45 seconds. After that, the temperature was maintained at 10 °C for a final elongation stage lasting 7 minutes at 72 °C.

The primer sequences for gene amplification were;

Forward; 16SrRNAF: 27F: AGAGTTTGATCMTGGCTCAG

Reverse; 16SrRNAR: 1525R: AAGGAGGTGWTCCARCCGCA.

The band size for the gene of interest was 1500 bp

For electrophoresis of DNA and PCR, one (1) g agarose was weighed using a digital balance (Labtech, BL2000l, USA). About 100 mL of 1xTAE were added to the material in a-safe flask. The agarose was totally melted after being steamed for 2 minutes. Over the course of 5 minutes, the solution was given time to cool to about 50°C. Subsequently, EZ vision DNA stain 10Ul was added to the mixture, binding to the DNA and enabling DNA viewing under UV lamp. With the well comb in place, the solution was poured onto a gel box and let to remain for 30 minutes until it had fully hardened.

The gel box was filled with 1x TAE (or TBE). A molecular weight ladder was carefully loaded into the gels first lane. Loading buffer was added to each of the DNA samples or PCR products**.** Samples were then loaded into the additional wells of the gel. The gel was run at 80-150 V for about 1 hour 30 minutes before the power was turned off and electrodes disconnected from the power source. The gel was removed from the box. PCR products were visualized with UV transilluminator.

DNA sequencing

The South African Medical Research Council in Cape Town, South Africa, used a 3510 ABI sequencer to perform the sequencing using the BigDye Terminator kit. The sequencing was done at a final volume of 10 µL; the components included 0.25 µL BigDye® terminator v1.1/v3.1, 2.25 µL of 5x BigDye sequencing buffer, 10 µM PCR primers AUG1 forward: 5'-CAATTTACATCTTTATTTATTAACG-3'; AUG1 reverse: 5'-GAAGAGAAAAACATTAGTTGGC-3' by Invitrogen and 2-10 ng PCR template per 100 bp. The following were the sequencing requirements: 96 °C for 10 seconds, 55 °C for 5 seconds, and 60 °C for 4 minutes are repeated 32 times.

BLAST analysis

BLAST analysis of the bacterial isolates was performed using the software RCBlast version 0.5.1.

RESULTS

Cultural characteristics of the bacterial isolates

Table 1's description of the physical traits of the bacterial isolates revealed that *Staphylococcus* spp had a creamy colony, was cocci in shape and had a convex elevation while *Escherichia coli* had a green metallic sheen with rod-like shape and a slightly raised elevation.

Table 1: Cultural characteristics of bacteria isolated from Pounded Yam sold by food vendors along major roads in Makurdi, Benue State

Colony colour	Margin	Colony shape	Elevation	Texture	Optical properties	Bacterial isolates
Green	Entire	Rod	Flat	Rough	Opaque	*Pseudomonas* spp
White	Irregular	Rod	Flat	Dry	Opaque	*Bacillus* spp
Yellow	Entire	Cocci	Convex	Dry	Shiny	*Staphylococcus* spp
Green metallic sheen	Entire	Rod	Slightly raised	Dry	Shiny	*E.* coli
Mucoid pink	Irregular	Rod	Convex	Mucoid	Opaque	*Klebsiella* spp
Grey	Entire	Rod	Slightly raised	Smooth	Shiny	*Proteus* spp

Biochemical characteristics of the bacterial isolates

As revealed by the biochemical characteristics of the bacterial isolates (Table 2), *Staphylococcus* spp tested positive to gram`s reaction, catalase, citrate, coagulase, indole and MR (methyl red test) but negative to urease, VP (Voges-Proskauer test), glucose, sucrose and lactose. *Escherichia coli* were gram negative and also negative to citrate, urease, coagulase, VP and sucrose but positive to catalase, indole, MR, glucose and lactose.

Table 2: Biochemical characteristics of bacteria isolated from Pounded Yam sold by food vendors along major roads in Makurdi, Benue State

Grams reaction	Catalase	Citrate	Urease	Coagulase	Indole	MR	VP	Glucose	Sucrose	Lactose	Bacterial isolates
-	+	+	-	-	-	-	-	-	-	-	*Pseudomonas* spp
+	+	+	-	-	-	-	+	+	+	-	*Bacillus* spp
+	+	+	-	+-	+	+	-	-	-	-	*Staphylococcus* spp
-	+	-	-	-	+	+	-	+	-	+	*E. coli*
-	+	+	+	-	-	+	-	+	+	+	*Klebsiella* spp
-	+	+	+	-	-	+	-	+	+	-	*Proteus* spp

Key: + = Positive, - = Negative

Percentage frequency of occurrence of the bacterial isolates across study locations

The percentage frequency of occurrence of the bacterial isolates across study locations as presented in Table 3 revealed that Pounded yam samples from Gboko accounted for the least percentage occurrence (11.98%), while samples from Wadata accounted for the highest percentage occurrence (17.19%). From the point of contaminating organisms, *Escherichia coli* accounted for 20.84% of contaminating bacteria implicated in this study followed by *Staphylococcus* specie (19.27%). Conversely, *Bacillus* specie had the least percentage occurrence accounting for 8.33% of the bacterial contaminant.

Table 3: Percentage frequency of occurrence of the bacterial isolates across study locations

	N(%)						
Locations	*Pseudomonas* spp	*Bacillus* spp	*Staphyloc-`occus* spp	*E. coli*	*Klebsiella* spp	*Proteus* spp	Total
Highlevel	2(1.04)	0(0.00)	6(3.13)	7(3.65)	3(1.56)	6(3.13)	24(12.50)
Otukpo	6(3.13)	3(1.56)	8(4.17)	6(3.13)	1(0.52)	3(1.56)	27(14.06)
Wurukum	4(2.08)	4(2.08)	4(2.08)	7(3.65)	5(2.60)	2(1.04)	26(13.54)
Wadata	8(4.17)	5(2.60)	3(1.56)	5(2.60)	3(1.56)	9(4.69)	33(17.19)
Gboko	6(3.13)	1(0.52)	3(1.56)	2(1.04)	6(3.13)	5(2.60)	23(11.98)
N/bank	4(2.08)	3(1.56)	6(3.13)	7(3.65)	4(2.08)	6(3.13)	30(15.63)
Naka	6(3.13)	0(0.00)	7(3.65)	6(3.13)	7(3.65)	3(1.56)	29(15.10)
Total	**36(18.75)**	**16(8.33)**	**37(19.27)**	**40(20.84)**	**29(15.10)**	**34(17.71)**	**192(100.00)**

The DNA of three bacteria isolated from pounded yam sold along major roads in Makurdi, Benue State and initially identified using biochemical characterization (in Table 2) was amplified at 1500bp using 16SrRNA gene amplification (Figure 1). The first lane, lane M, contained the molecular weight ladder while the second lane, lane 1, constituted *Staphylococcus aureus*. The third lane, lane 2, contained *Staphylococcus sciuri* while the fourth lane, lane 3, constituted *Escherichia coli*. The primer generated the amplicon at 1500 base pair.

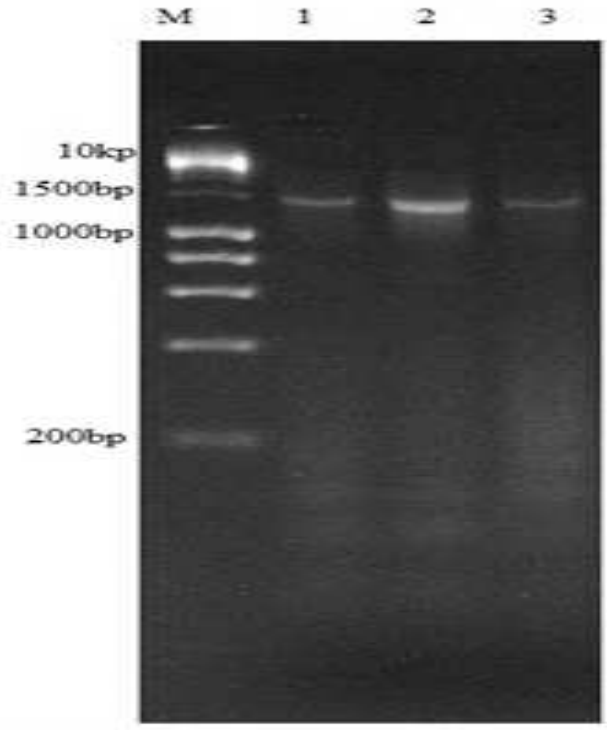

Figure 1: 1500bp 16SrRNA gene amplification of *Staphylococcus aureus* (Lane 1), *Staphylococcus sciuri* (Lane 2) and *Escherichia coli* (Lane 3) isolated from pounded yam sold along major roads in Makurdi, Benue State, with Lane M containing the Molecular weight ladder.

BLAST analysis of bacterial contaminants from pounded yam

The result of the BLAST analysis of the bacterial contaminants from pounded yam as shown in Table 4 revealed that *Staphylococcus* spp 1 had 93.80 % pairwise identity on *Staphylococcus aureus* strain B3A22 with NCBI accession number KX023358 and E value of 0. *Staphylococcus* spp 2 had 93.10 % pairwise identity on *Staphylococcus sciuri* strain LZH-22 with NCBI accession number MN121253 and E value of 0. *E. coli* sample had 76.70 % pairwise identity on *Escherichia coli* strain G1F12 with NCBI accession number GU646051 and E value of 1.29E-05.

Table 4: Result of the BLAST analysis of bacterial contaminants from pounded yam

Sample	Strain	NCBI accession number	Percent pairwise identity	E value	Bacterial isolate
Staphylococcus spp 1	B3A22	KX023358	93.80	0	*Staphylococcus aureus*
Staphylococcus spp 2	LZH-22	MN121253	93.10	0	*Staphylococcus sciuri*
E. coli	G1F12	GU646051	76.70	1.29E-05	*E. coli* G1F12

DISCUSSION

Morphological and biochemical characteristics of the bacterial isolates revealed six genera of bacteria implicated as contaminants of pounded yam. They include; *Escherichia coli, Klebsiella* spp, *Bacillus* spp*, Staphylococcus* spp, *Proteus* spp *and Pseudomonas* spp. The percentage frequency of occurrence of the bacterial isolates revealed *Escherichia coli* occurred the most (20.84 %), followed closely by *Staphylococcus* spp (19.27 %). *Bacillus* spp had the least percentage occurrence (8.33 %). This is in line with Muhammad *et al.* (2016), which identified that *Staphylococcus aureus* occurred the most (42.3 %), followed closely by *Escherichia coli* (40.8 %). He also identified *Bacillus cereus* with the least percentage occurrence (4.2 %) in ready-to-eat (RTE) foods. Across locations, Wadata had the highest bacterial contamination (17.19 %) while Gboko road had the least contamination (11.98 %). This implies that the pounded yam from Gboko road were prepared under better hygiene when compared to other locations. Otukpo road samples had the highest contamination with *Staphylococcus* spp (4.17 %), followed by Naka road samples (3.65 %) which might have been caused by the busy and dusty nature of these roads. The dust particles harboring the bacteria might have settled on the pounded yam contaminating it. Highlevel, Wurukum and Northbank were highest in *Escherichia coli* (3.65 %) confirming the pounded yam were prepared under unhygienic conditions. These results are consistent with earlier research by Zumbes *et al.* (2014), who discovered various bacterial species in various areas in Jos, Nigeria.

The BLAST analysis of the bacterial DNA sequences confirmed some of the contaminating bacterial species in the pounded yam samples as *Escherichia coli* strain G1F12, *Staphylococcus aureus* strain B3A22 and *Staphylococcus sciuri* strain LZH-22. The presence of enteric bacteria like *E. coli* in the pounded yam leaves much to be desired in terms of personal hygiene of the handlers of this food and the type of water used for the preparation of the pounded yam. Also, the presence of *Staphylococcus aureus* which are pathogenic bacteria is of public health importance because it could lead to a disease outbreak. *Staphylococcus sciuri* is also a pathogenic bacterium of animal origin as reported by Meservey *et al.* (2020). The bacteria implicated in the contamination of pounded yam in this study agrees with previous works of Maifreni *et al.* (2013), Kabiru *et al.* (2013), Oranusi *et al.* (2013) and Muhammad *et al.* (2016) who also isolated similar bacteria from cheese, garri, cassava, foo foo and other ready-to-eat (RTE) foods. The presence of these organisms in the roadside pounded yam makes it unsafe for human consumption. *Escherichia coli* for instance, are indicative of faecal contamination as also reported by Odonkor and Ampofo (2013). It may have entered through the water used in washing or cooking the yam, or even from the plates used for dishing the food. This route of entry correlates with the findings of Bintsis (2017). There are generally poor hygienic practices by those involved in handling of this food, as such; the handlers may also be a route of entry. *Staphylococcus aureus* and *Staphylococcus sciuri* can be air-borne implying that the route of entry may be due to the exposure of the pounded yam to the air or environment. The customers may also contribute to the contamination by releasing these organisms through their breath. The released bacteria become concentrated in the eating environment because of the congestion from insufficient ventilation. *Staphylococcus sciuri* was found on the skin and mucosal surfaces of farm animals, as such, the type of meat used in preparing the food could have been an entry route for contamination of the pounded yam if not washed and cooked properly. Kengkoom and Ampawong (2017) isolated *Staphylococcus sciuri* from rodents of which the bush meat popularly called “Nyongu” in Tiv dialect; together with pork happens to be the favorite meat of the locals. Thus the

pounded yam was also exposed to contamination if such meat harboring the bacteria was used in preparing the food. This spread of food-borne pathogens is of public health concern because it can result to disease outbreaks which may bear extreme consequences on the public.

CONCLUSION

The bacterial species implicated in this study include; *Escherichia coli* strain G1F12, *Staphylococcus aureus* strain B3A22 and *Staphylococcus sciuri* strain LZH-22 which are easily transported to food. These bacteria have been implicated as the cause of some food-borne diseases but despite the high risk of food-borne diseases associated with roadside foods, its preparation, sale and consumption is still on the rise. These foods are often prepared under unhygienic conditions by food vendors many of whom are uneducated on the causes and prevention of food-borne diseases. Hygienic method of food preparation needs to be practiced to prevent the contamination of roadside foods with these bacteria.

RECOMMENDATIONS

From the findings of this research, we made the following recommendations. First, there is the need for public awareness on good hygienic practices and proper ways of handling ready-to-eat (RTE) food substances of which pounded yam is one. Second, proper storage facilities that could minimize bacterial contamination of food should be made available. Third, more research is recommended to determine the route of entry of pathogen and possible preservation method(s) that will not affect the nutritional as well as the organoleptic properties of this food.

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