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Determination of Bacterial Isolates Associated with Fruits Spoilage in Gwagwalada Market, Abuja Nigeria

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Abstract

The research was conducted to isolate and characterize various bacterial populations associated with fruits spoilage in Gwagwalada market Abuja, Nigeria. Seven different most commonly consumed fruits viz: oranges (*Citrus sinensis*), Tomatoes (*Lycopersicon lycopersicum*), Banana (*Musa* spp.), Mango (*Mangifera indica*), Red pepper (*Capsicum* sp), Guava (*Psidium guajava*) and Water melon (*Citrullus lanatus*) were used for the study. The fruits that showed decayed symptomatology were collected and transported in separate polythene bags to the laboratory where the rotten parts were isolated, cultured on four bacteriological media: Mannitol Salt Agar (MSA), MacConkey Agar (MA), Salmonella Shigella Agar (SSA) and Blood Agar (BA). Characterization of the isolates was done on the basis of cultural characteristics and biochemical tests. The result obtained revealed eight different bacterial species associated with the spoilage of fruits in Gwagwalada market. These bacteria are *Staphylococcus aureus* (25%), *Escherichia coli* (17%), *Bacillus* (19%), *Klebsiella* (10%), *Pseudomonas* (10%), *Lactobacillus* (10%), *Micrococcus* (07%) and *Salmonella* (07%). This implies that consumption of such spoilt fruits renders the risk of certain clinical symptoms to the consumers. Therefore people should avoid consumption of such spoilt fruits.

Key Words: *Abuja; Bacteria; Characterization; Isolation; Spoilage*

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Introduction

Most of our foods are excellent source for rapid microbial growth. Food materials contain organic substances in plenty and sufficient amount of water, and they may be either neutral or slightly acidic in nature [1]. They are subjected to natural contamination by many different kinds of microorganisms, including pathogens. Metabolic activities of microbes alter the condition of food, resulting in its spoilage [2]. The airborne microbes fall on fruits and vegetables and enter through the ruptured skin. The microorganisms present in the soil reach the processing plant through the crops [3]. Several insects are also responsible for the transference of microbes to the food. In general, the keeping quality of food depends on the success of preventing the entry of micro-organisms and restricting their growth [1]. Differences in microbial profiles of various fruits result largely from unrelated factors such as resident micro flora in the soil, application of non-resident micro flora via animal manures, sewage or irrigation water, transportation and handling by individual retailers [4].

In developing countries such as Nigeria, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contaminations [5,6]. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries. It has been known that fruits constitute commercially and nutritionally important indispensable food commodity [7]. Fruits are highly perishable products; the quality is affected by post-harvest handling, transportation, storage and marketing. The improper handling, packaging, storage and transportation may result in decay and production of microorganisms, which become activated because of the changing physiological state of the fruits [8].

Microorganisms especially bacteria and fungi have been identified as major organisms causing deterioration of various fruits by the secretion of extracellular cell wall degrading enzymes [9]. Most of the reported outbreaks have been associated with bacterial contamination, particularly members of the Enterobacteriaceae [10]. A large number of lactic acid bacteria, coliforms, moulds and yeast have been reportedly implicated in food spoilage as they use the carbohydrate content of the foods for undesirable fermentation processes [11]. The microorganisms normally present on the surface of raw fruits may consist of chance contaminant from the soil or dust. These include bacteria or fungi that have grown and colonized by utilizing nutrient exuded from plant tissue. Among the group of bacteria commonly found include faecal coli forms such as *Klebsiella* and *Enterobacter* [12]. The study was aimed to determine bacterial species associated with spoilage of some fruits (oranges (*Citrus sinensis*), Tomatoes (*Lycopersicon lycopersicum*), Banana (*Musa* spp.), Mango (*Mangifera indica*), Red pepper (*Capsicum* sp), Guava (*Psidium guajava*) and Water melon (*Citrullus lanatus*) in Gwagwalada market, Abuja Nigeria

Materials and Methods

Study Area

The Study area is Gwagwalada market which is part of Abuja Municipal Area Council (AMAC). The AMAC is located between latitude 8°40' and 9°20' North of the equator and longitude 6°40' and 7°40' east of the Greenwich meridian. The Abuja Federal Capital Territory (FCT) has a land mass of approximately 8000sq km of which the FCC occupies about 250sq km with population recent census at 778,567 for Abuja Municipal Area Council [13].

Samples Collection

A total seventy (70) samples, ten (10) each of orange, tomato, banana, mango, pepper, guava and water melon were purchased from different vendors within Gwagwalada market Abuja Nigeria. The spoiled samples were taken to the laboratory for use. The decaying portions were subjected for bacterial identification [14]. The sample names were coded for easy reference on the basis of their types (Table 1).

S/N	Fruit	Code
1	Orange	O
2	Tomato	T
3	Banana	B
4	Mango	M
5	Pepper	P
6	Guava	G
7	Watermelon	W

Table 1: *Samples name and their code.*

Preparation of Media

The composition of the supplied agar medium was 28g per 1000 ml and 38 grams per 1000ml for Nutrient agar (NA), per 100 for Mac Conkey agar, 51.5 grams per 1000ml for Salmonella–Shigella agar and 40 gram per 1000ml for Blood agar, thus the amount required was weighted and taken in to reagent bottle and used to prepare 1000 ml of agar solution. Then distilled water was added up to 1000 ml in accordance with manufacturer's instruction and the reagent bottle was sterilized in autoclave at a temperature of 121ºc for 15 minutes at about 1.30 hours [15].

Isolation of Bacteria

Five grams (5g) of decayed portion from each sample was weighed and washed in 10 ml of sterile distilled water. MacConkey, Nutrient agar, Salmonella–Shigella agar and Blood agar were inoculated with 0.1 ml of the rinse water using the Pour Plate Technique. The plates were allowed to solidify, inverted and incubated at 37ºC for 24h for colony formation. Each colony was isolated in a pure form by sub culturing for further studies and identification. Distinctive morphological properties of each pure culture such as colony form, elevation of colony and colony margin were observed. Further microbial identification was based on the methods of Holt., *et al.* [16].

Bacteria Identification

The distinct colonies that develop in the pure culture plate were observed for the morphological and cultural characteristics including the nature of margin, elevation, shape, colour and transparency and Gram staining [16-18] and set of Biochemical Characterization i.e. indole test, Methyl-Red test, Vogues-Proskauer test and Citrate utilization test, catalase test, coagulase test and oxidase test by standard method given by Sherman [17] and Holt., *et al*. [16].

Results

Morphological Characterization of the Isolates

The morphological characteristics of the recovered isolates are presented in (Table 2). Total of twenty nine (29) isolates were characterized on the basis of colony morphology and the staining characteristics. It was found that 16 isolates were Gram negative while 13 isolates were Gram positive.

	S/code	I/code	G/staining	Shape	Cultural morphology
1	O	O_1	+	Coccus	Produce opaque cream yellow growth
2	O	O_2	+	Rod	Produce large irregular flat growth
3	O	O_3	-	Rod	White glistering and moist growth
4	O	O_4	+	Rod	Small, creamy, whitish, convex colonies
5	T	T_1	-	Rod	White glistering and moist growth
6	T	T_2	-	Rod	Produce colorless colony
7	T	T_3	+	Cocci	Produce opaque cream yellow growth
8	T	T_4	+	Rod	Produce large irregular flat growth
9	B	B_1	-	Rod	White glistering and moist growth
10	B	B_2	-	Rod	Produce shiny mucoid/viscous colony
11	B	B_3	-	Rod	White growth turning media light green
12	B	B_4	+	Coccus	Produce opaque cream yellow growth
13	M	M_1	+	Coccus	Produce opaque cream yellow growth
14	M	M_2	-	Rod	White glistering and moist growth
15	M	M_3	-	Rod	Produce shiny mucoid/viscous colony

16	M	M_4	+	Rod	Produce large irregular flat growth
17	M	M_5	+	Rod	Small, creamy, whitish, convex colonies
18	P	P_1	+	Coccus	Produce opaque cream yellow growth
19	P	P_2	-	Rod	White growth turning media light green
20	P	P_3	+	Coccus	Forms opaque smooth yellow growth
21	G	G_1	-	Rod	Produce colorless colony
22	G	G_2	-	Rod	Produce shiny mucoid/viscous colony
23	G	G_3	+	Coccus	Produce opaque cream yellow growth
24	G	G_4	+	Rod	Small, creamy, whitish, convex colonies
25	C	C_1	+	Coccus	Produce opaque cream yellow growth
26	W	W_2	-	Rod	White glistering and moist growth
27	W	W_3	-	Rod	White growth turning media light green
28	W	W_4	+	Coccus	Forms opaque smooth yellow growth
29	W	W_5	+	Rod	Produce large irregular flat growth

Table 2: *Morphological characteristics of the recovered isolates.*

Key: S/code = Sample code, I/code = Isolate code, G/staining = Gram staining

Biochemical characterization of the isolates

The biochemical characterization of the recovered isolates is presented in (Table 3). Total of thirty four isolates were characterized based on indole, methyl-red, Vougues Proskeaur, citrate utilization, catalase, coagulase, oxidase and motility tests. Nitrate, lactose and mannitol fermentation were also conducted.

S/N	S/code	I/code	IN	MR	VP	CU	CA	CO	OX	MO	NR	LF	MF	Organisms identified
1	O	O_1	-	+	+	+	+	+	-	-	+	+	+	*Staphylococcus aureus*
2	O	O_2	-	-	+	+	+	-	-	+	-	-	-	*Bacillus cereus*
3	O	O_3	+	+	-	-	+	-	-	+	+	+	+	*Escherichia coli*
4	O	O_4	-	-	-	-	-	-	-	-	+	+	+	*Lactobacillus sp*
5	T	T_1	+	+	-	-	+	-	-	+	+	+	+	*Escherichia coli*
6	T	T_2	-	+	-	+	+	-	-	-	+	+	-	*Salmonella typhi*
7	T	T_3	-	+	+	+	+	+	-	-	+	+	+	*Staphylococcus aureus*
8	T	T_4	-	-	+	+	+	-	-	+	-	-	-	*Bacillus cereus*
9	B	B_1	+	+	-	-	+	-	-	+	+	+	+	*Escherichia coli*
10	B	B_2	-	-	+	+	+	-	-	-	+	+	+	*Klebsiella sp*
11	B	B_3	-	-	-	+	+	-	+	+	+	+	-	*Pseudomonas aeruginosa*
12	B	B_4	-	+	+	+	+	+	-	-	+	+	+	*Staphylococcus aureus*
13	M	M_1	-	+	+	+	+	+	-	-	+	+	+	*Staphylococcus aureus*
14	M	M_2	+	+	-	-	+	-	-	+	+	+	+	*Escherichia coli*
15	M	M_3	-	-	+	+	+	-	-	-	+	+	+	*Klebsiella sp*
16	M	M_4	-	-	+	+	+	-	-	+	-	-	-	*Bacillus cereus*
17	M	M_5	-	-	-	-	-	-	-	-	+	+	+	*Lactobacillus sp*
18	P	P_1	-	+	+	+	+	+	-	-	+	+	+	*Staphylococcus aureus*

19	P	P_2	-	-	-	+	+	-	+	+	+	+	-	*Pseudomonas aeruginosa*
20	P	P_3	-	-	-	+	+	-	+	+	+	-	-	*Micrococcus sp*
21	G	G_1	-	+	-	+	+	-	-	-	+	+	-	*Salmonella typhi*
22	G	G_2	-	-	+	+	+	-	-	-	+	+	+	*Klebsiella sp*
23	G	G_3	-	+	+	+	+	+	-	-	+	+	+	*Staphylococcus aureus*
24	G	G_4	-	-	-	-	-	-	-	-	+	+	+	*Lactobacillus sp*
25	W	W_1	-	+	+	+	+	+	-	-	+	+	+	*Staphylococcus aureus*
26	W	W_2	+	+	-	-	+	-	-	+	+	+	+	*Escherichia coli*
27	W	W_3	-	-	-	+	+	-	+	+	+	+	-	*Pseudomonas aeruginosa*
28	W	W_4	-	-	-	+	+	-	+	+	+	-	-	*Micrococcus sp*
29	W	W_5	-	-	+	+	+	-	-	+	-	-	-	*Bacillus cereus*

Table 3: *Biochemical characteristics of the recovered isolates.*

Key: S/code = Sample code, I/code = Isolate code, SH = Shape, IN = Indole, MR = Methyl-red, VP = Vougues Proskeaur, CU = Citrate Utilization, CA = Catalase, CO = Coagulase, OX = Oxidase, MO = Motility, NR = Nitrate reduction, MF = Mannitol fermentation, LF = Lactose fermentation

Frequency of bacteria isolates

The frequency of bacteria isolates associated with spoilage of fruits in Gwagwalada market Abuja, Nigeria is presented in (Table 4). The percentage occurrence of the organisms is as follows; *Staphylococcus aureus* (25%), *Escherichia coli* (17%), *Bacillus* (19%), *Klebsiella* (10%), *Pseudomonas* (10%), *Lactobacillus* (10%), *Micrococcus* (07%) and *Salmonella* (07%).

Isolates	Frequency	Orange	Tomato	Banana	Mango	Pepper	Guava	WMelon
S. aureus	7 (25%)	1	1	1	1	1	1	1
E. coli	5 (17%)	1	1	1	1	0	0	1
Bacillus	4(14%)	1	1	0	1	0	0	1
Micrococcus	2 (07%)	0	0	0	0	1	0	1
Klebsiella	3 (10%)	0	0	1	1	0	1	0
Lactobacillus	3 (10%)	1	0	0	1	0	1	0
Pseudomonas	3 (10%)	0	0	1	0	1	0	1
Salmonella	2 (07%)	0	1	0	0	0	1	0
Total	29 (100)	4	4	4	5	3	4	5

Table 4: *Frequency and percentage occurrence of bacterial isolates.*

Discussion

The colonization of fruits by the invading microorganism is a critical phase in the microbial spoilage of produce. The colonization process involves the ability of the microorganism to establish itself within the produce. This is initiated when the microorganism degrade certain specific cell wall polymers such as prospecting, the cementing substance of the fruits following adhesion and release of enzyme. The magnitude of the symptoms of the induced disease is a reflection of the extent of colonization [19].

The study was aimed to determine bacteria isolates responsible for spoilage of various fruit at Gwagwalada market, Abuja Nigeria. A total of twenty nine (29) bacteria isolates were recovered which are grouped into 7 species. The bacterial isolates were characterized

on the basis of colony morphology, staining characteristics and biochemical test. It was observed that 17 isolates were Gram positive, while 12 isolates were Gram negative. The result obtained from the data shows that the bacteria found in spoiled fruits samples include; Staphylococcus aureus (25%), *Escherichia coli* (17%), *Bacillus* (19%), *Klebsiella* (10%), *Pseudomonas* (10%), *Lactobacillus* (10%), *Micrococcus* (07%) and *Salmonella* (07%) and. This shows Staphylococcus was the highest occurring organism and found in all the fruit samples tested. The detection of the bacteria isolates in this study showed poor hygienic standard in handling of the fruits, it could be also be from contamination during harvest. Most of the organisms found in this study are those commonly found in soil and water. But the presence of other indicator organisms like *E. coli*, *Salmonella* and *Klebsiella* in those vegetable samples might be the result of possible contamination during sales or unhygienic handling of stored fruits. The presence of *Escherichia coli, Salmonella* and *Klebsiella* in this study is an indication of faecal contamination of the food as a result of possible unhygienic handling [20].

The high microbial contamination observed in this study may also be a reflection of storage conditions and how long these produces were kept before they were obtained for selling. More importantly, bacteria on the produce may multiply over time depending on the storage conditions [21]. Several studies were conducted on isolation and characterization of microorganisms responsible for spoilage of fruits. The finding of this study was in conformity with that of Kumar., *et al.*. [22] on Isolation and Characterization of Microorganisms Responsible for Different Types of Food Spoilages The result obtained from their study shows that the bacteria found in spoiled fruits samples was *Bacillus, Klebsiella, Pseudomonas, E. coli, Lactobacillus, Staphylococcus, Micrococcus* and there prevalence was 18%, 15%, 14%, 10%, 7%, 5%, 4% respectively. The result of this study justifies the findings of Aminu., *et al.* [23] on isolation and identification of microorganisms associated with spoilage of watermelon (*Citrus lanatus*) In Sabon-Gari Market Kano Nigeria. Their result shows that Bacillus cereus (23%) were the most predominant bacterial isolates associated with spoilage of water melon tested, followed by Staphylococcus aureus and *Salmonella* sp (21%) each, *Escherichia coli* (19%), while *Klebsiella* spp. and Micrococcus were least predominant with 12% and 10% respectively.

Conclusion

Conclusively, the results of the present study revealed that the spoiled fruits (orange, tomato, banana, mango, pepper, and guava and water melon) samples are mainly contaminated with B*acillus cereus, Staphylococcus aureus*, *Salmonella* sp, *Escherichia coli*, *Lactobacillus*, *Klebsiella* and *Micrococcus*. Presence of these bacteria on fruits most especially Coliforms pose a serious threat to health of consumers as the organism could produce toxins, which are lethal when consumed. It is therefore necessary and important that both the farmers and sellers to take necessary and appropriate precautions in preventing contamination and eating of contaminated fruits. This will however reduce the risk of toxins associated with bacterial contamination which are deleterious to human health.

References

1. Singh V., *et al*. "A text book of botany". Fourth Edition, Rastogi publication (2013).
2. Angela O., *et al*. "Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria". *African Journal of Food Science* 4 .5 (2010).
3. Neeraj D and Sharma S. "Food spoilage food infection and intoxication caused by microorganism and method of their detection". *Food and Industrial Microbiology* (2007).
4. Ray B and Bhunia AK. "Fundamental Food Microbiology 4th Edition CRC Press, USA. (2007): 492.
5. Olayemi AB. "Microbiological hazard associated with agricultural utilization of urban polluted river water". *Intern. Journal environmental health research* 7.2 (2007): 149-154.
6. Amoah P., *et al*. "Improving food hygiene in Africa where vegetables are irrigated with polluted water". *Regional sanitation and hygiene symposium Accra, Ghana* (2009).
7. Al-Hindi RR., *et al*. "Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes". *African Journal of Microbiology Research* 5.4 (2011): 443-448.
8. Wilson CL., *et al*. "Biological control of post-harvest diseases of fruits and vegetables: alternative to synthetic fungicides". *Crop Protection* 10: (199): 172-177.

9. Ayanda OI., *et al*. "Isolation, characterization and extracellular enzyme detection of microbial isolates". *International Journal of Biological and Chemical Sciences* 7.2 (2013): 641-648.
10. Verma S and Srivastav G. "Original Article Isolation and Characterization of Microorganisms". *International Journal of Research in Pure and Applied Microbiology* 1.2 (2011): 22-31.
11. Essien E., *et al*. "Evaluation of the Nutritional and Microbiological Quality of Kunun (A Cereal Based Non-Alcoholic Beverage) in Rivers State, Nigeria". *The Internet Journal of Nutrition and Wellness* 10.2 (2011).
12. Anitha M., et al. "Prevalence of disease causing microorganisms in decaying fruits with analysis of fungal and bacterial species". *International Journal of Research in Health Sciences* 2.2 (2014): 547-554.
13. National Population Commission (NPC). National population census result, Abuja Nigeria (2006).
14. Anita T. "Food and Nutrition 3rd Edition Oxford Press" (1997).
15. Prescott LM., *et al*. "Microbiology, 5th Edi, McGraw-Hill', New York. (2002): 1014.
16. Holt JG., *et al*. "Bergey's Manual of Determinative Bacteriology 9th Ed". Baltimore Md Williams and Wilkins Co. Baltimore, Maryland, (1994): 786.
17. Sherman N. "Microbiology: A laboratory manual. Sixth Edition". *ISBN* 81.3 (2005): 265-267.
18. Chessbrough M. "District laboratory practice in tropical countries. Second edition, part two, Cambridge university press". *Examination of pus, ulcer material and skin specimens* (2006): 80-85.
19. Chuku EC., *et al*. "Comparative Studies on the Fungi and Bio-Chemical Characteristics of Snake Gaurd (Trichosanthes curcumerina linn) and Tomato (Lycopersicon esculentus mill) in Rivers state, Nigeria". *Journal of Applied Sciences* 8.1 (2008): 168-172.
20. Okonko IO., *et al*. "Hazards analysis critical control points (HACCP) and Microbiology qualities of Sea-foods as affected by Handler's Hygiene in Ibadan and Lagos, Nigeria". *African Journal of Food Sciences* 3.1 (2009): 035-050.
21. Montville TJ and Mathews KR. "Food Microbiology: Introduction 2nd EDITION". American Society of Microbiology (ASM) Press (2008).
22. Kumar A., *et al*. "Isolation and Characterization of Microorganisms Responsible for Different Types of Food Spoilages". *International Journal of Research in Pure and Applied Microbiology* 1.2 (2011): 22-31.
23. Aminu F., *et al*. "Assessment of Micro-organisms Associated with Spoilage of Watermelon (Citrillus lanatus) in Kano, Nigeria". *International Journal of Applied Research and Technology* 6.2. (2017): 55-61.