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Microbial Flora Associated with Postharvest Spoilage of White Yam (Dioscorea rotundata) and Implication for Health of Consumers in a Canteen in Ibadan

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Abstract
The microbial flora responsible for postharvest spoilage in white yam **(***Dioscorea rotundata*) was investigated. The yams were collected from a canteen in Ibadan, where a suspected food poisoning was reported. The yams were partly discoloured, partly rotten and termites have nested in some portions. The predominant flora identified were fungi, aerobic bacteria viz. *Bacillus sp., Pseudomonas sp, Staphylococcus sp., and Micrococcus sp*, and enteric bacteria, viz. *Escherichia coli, Proteus sp., and Aeromonas sp. However, there was no Clostridium sp.* The fungi *Candida* and *Geotricum* species were found common in all the portions of the yam samples. The termite infested portion (T) contained in addition, *Aspergillus* sp. and *Penicillium sp*, the rotted portion (R) contained *Aspergillus sp*, while the discoloured portion contained *Neurospora sp.*. The study emphasized the need for proper postharvest storage for tuberous crops, proper selection of tubers for food preparation particularly in canteens where many people eat. Hazard Analysis Critical Control Point (HACCP) System may be used in canteens to avoid any poisoning arising from farms.
Keywords: Fungi, microbial flora, postharvest spoilage, white yam **(***Dioscorea rotundata*)

INTRODUCTION

Diseases caused by foodborne pathogens constitute a worldwide public health problem and preventing them is a major goal of societies. Microbiological foodborne diseases are typically caused by bacteria or their metabolites, parasites, fungi, virus or toxins (The International Commission on Microbiological Specifications for Foods, ICMSF (2006).

Yams (*Dioscorea* spp) are root crops which are grown for their edible tubers. They provide the staple carbohydrate food in many parts of West Africa. Nigeria alone produces 21,814 million tons of yams per year, making it the world's largest yam producer (Okigbo, 2004). This region accounts for over 60% of the world yam production. Yam has many important cultural values attached to it, especially during weddings and other social and religious ceremonies. In many farming communities in Nigeria and other West African countries, the size of yam enterprise that one has is a reflection of one's social status. Due to the importance attached to yam, many of these communities celebrate yam festival annually (Awoniyi et al, 2006). Yam is eaten in various forms, cooked or boiled, fried, pounded etc. and is a staple food in the menu of many Nigerians.

Lack of good storage and processing facilities cause a lot of wastage of agricultural produces such as tubers, roots, pulses, fruits and vegetable crops. Specifically, storage is one of the critical problems limiting yam production. According to Okigbo (2004), an estimate of 56% of yam is lost to rot after six months of storage. These losses are due to pathological problems brought about by bacteria, fungi and nematodes (Booth, 1974).

Roots and tubers bruised or otherwise damaged during harvesting may undergo early infestation with moulds and viruses which may in turn lead to rotting. In most countries, several traditional methods of storage are in use. However, losses in these types of storage are very high and may be attributed to a number of factors such as: infestation of yam by *Scutellonema* (yam nematode), fungi or bacterial rots, rodents, insects, or physiological losses due to sprouting and respiration (FAO, 1985). An assessment of mycoflora and occurrence of aflatoxin B_1 in dried yam chips by Bankole and Mabekoje (2004) showed that *Aspergillus* and *Penicillium* were the two prevalent genera of fungi, and that the number of colony forming units of the two genera in the yam chips studied exceeded the tolerance limits in foodstuffs specified by the International Commission on Microbiological Specifications for Foods (ICMSF) 1996. This study assessed the microbial flora in white yam **(***Dioscorea rotundata*) that was implicated in causing food poisoning at an institutional food canteen in Ibadan, Nigeria

MATERIALS AND METHODS

This study was exploratory and laboratory based. It was as a consequence of a food poisoning outbreak in an

institutional food canteen in Ibadan, Nigeria. Two white yam (*Dioscorea rotundata*) tubers belonging to 2013 early harvest were randomly selected from a batch of yams supplied to the canteen.

Culture media and chemicals
The culture media used were peptone water, Czapek Dox agar (for fungi), Nutrient agar (for total microbial load), and MacConkey agar (for enteric bacteria-coliforms). These and other chemicals used were of analytical grade and were obtained from the laboratory of the Department of Environmental Health Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Physical examination
The two samples (A and B) selected for analysis were physically examined for damage, spoilage and discolouration.

Sample collection and preparation
Yam Samples were divided into different portions and tagged: Whole portion (W), Termite infested region (T), Rotted portions (R) and Discoloured portions (D).

Chemical analysis
The different portions of the two samples A and B were eluted with petroleum ether. Paper chromatography of the petroleum ether extracts of the four portions of each sample, W, T R and D was carried out and compared with pyrethrin family of pesticides (namely Cyfluthrin/Transfluthrin and Cypermethrin) which are commonly used by Nigerian farmers in agriculture and commodity storage.

Microbiological analysis
One gram of each portion was macerated and homogenized with sterile water. The microbial flora was assessed by plating 1ml of 1:10 dilution of each sample on Czapek Dox agar, Nutrient agar, and MacConkey agar according to standard procedures (Jay, 1978; Olutiola at al., 1991). Culture media were those of Oxoid and Difco.

Appropriate serial dilution of all the samples was carried out and 1 ml of the selected dilution was added to 20ml of sterile medium in duplicate Petri-dishes. This technique was used for the enumeration of total aerobic viable count, coliforms, fungal and Clostridium counts on Nutrient Agar and MacConkey Agar, Czapek Dox agar and Reconstituted Clostridial Medium, respectively. All cultures for bacteria were incubated at 37°C for 24 hrs except for fungi which were incubated at 28-30°C for 3 to 5 days. The organisms were purified by sub-culturing and then identified to the genera level. Media used were prepared according to the manufacturers' instructions.

RESULTS AND DISCUSSION

Physical observation
Physical examination showed that the two batches of yams belonged to 2013 early harvest. A lot of spoilage, rot and discolouration had taken place on the tubers to the extent that termites had made temporary nests on them. The presence of termites inside the crevices of the yam tubers suggest that the yams represent a bio-friendly environment as the rot progressed from outside to the inside.

Chemical Examination
Results of paper chromatography showed that the yam samples did not contain any extract with RF values consistent with those of the possible pesticides used. Subject to more precise analysis, it can be concluded that the portions of the two yam samples did not contain these pesticides which are normally used on yam farms.

Microbiological analysis
The results of microbiological analysis carried out on different portions of the samples A and B are shown in Table 1, while Table 2 gives the names of specific microorganisms observed from samples collected from different portions of the yam tubers. The rotted portions (R) in the two samples A and B had the highest mean fungi count ($3x10^4$ cfu/g); aerobes ($3x10^4$ cfu/g), and coliform bacteria ($2x10^4$ cfu/g). A count of ($8x10^3$ cfu/g) obtained from the Reconstituted *Clostridium* medium *was* not *Clostridium* sp but yeast. *Candida* and *Geotricum* species were the two prevalent genera of fungi found in the different portions of the yam samples. However, in addition to these two fungi, the termite infested portion (T) contained *Aspergillus* and *Penicillium*, the rotted portion (R) contained *Aspergillus*, while the discoloured portion contained *Neurospora*. Also, only the rotted portion contained *Flavobacterium* in addition to other aerobes present while both the termite infested and rotted portions contained *E. coli.* No *Clostridium* spp. was isolated from the Reconstituted Clostridial Medium but yeasts grew on the medium. Fig. 1 shows the cultures obtained from the samples on various media used. In line

with the results obtained in this study, Oyelana et al., 2011 isolated eight (8) fungal species which included ***Aspergillus flavus***, *A. niger*, *Botryodiploidia theobromae,* ***Fusarium oxysporum***, *F. solani*, *Penicillium chrysogenum*, *P. oxalicum* and *Rhizopus stolonifer* and two (2) bacterial species viz; *Pseudomonas* spp. and *Klebsiella* spp. from the rot portions of tubers of *Dioscorea rotundata*.

Results in Figure one show that Aeromonas spp was detected in most of the sample portions except D (Discoloured portions). According to ICMSF (1996b), illness due to *Aerommonas* spp can range from a mild diarrhoea to a life-threatening, cholera-like disease. Even though *Aeromonas* spp have not been clearly demonstrated as responsible for an outbreak of gastrointestinal illness, all species have been isolated and epidemiologically linked to this disease (ICMSF, 1996) However, case report of an oncological patient by Grobner et al., (2007) revealed that *Aeromonas veronii* biovar sobria was isolated as the causative enteropathogen of diarrhoea after failure of detection of other infectious agents.

Study by Babajide et al (2006) showed that there was predominance of *Staphylococcus aureus*, fungi and coliforms in the "Gbodo" samples prepared from yam tubers. The total viable count, fungi count and *S. aureus* count from the different locations ranged from 7.8 x 10^5 cfu/ml to 1.1 x 10^6 cfu/ml; 8.5 x 10^5 cfu/ml to 1.2 x 10^6 cfu/ml; and .5 x 10^3 cfu/ml to 9.0 x 10^4 cfu/ml respectively. The study concluded that the levels of contamination of the collected samples were high, even when compared with international standards (ICMSF) of 1996.

A study of the potential of isolates of *Bacillus subtilis* from yam farm soil to control rot of yam in storage barns by Okigbo (2005) showed that yam tubers inoculated *in vivo* with *B. subtilis* showed no rot while those inoculated with *Aspergillus niger, Botryodiploidia theobromae* or *Penicillium oxalicum* showed considerable rot.

Foods that need to be cooked before consumption may contain harmful bacteria that can contaminate other foods in a kitchen. Reducing the likelihood of cross-contamination from these products could be important in achieving a public health goal.(ICMSF, 2006)

Table 1: Total bacteria and fungal count of the organisms isolated from the samples

Portion studied	Czapek Dox agar		Aerobic Plate Count (cfu/g)		*Clostridium* sp		Coliform bacteria (cfu/g)	
	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
W	$1x10^4$	$1x10^4$	$1x10^4$	$1x10^4$	$4x10^3$	$5x10^3$	$1x10^3$	$1x10^4$
T	$3x10^3$	$3x10^3$	$2x10^4$	$2x10^4$	$2x10^3$	$1x10^3$	$1x10^3$	$2x10^3$
R	$3x10^4$	$3x10^4$	$3x10^4$	$3x10^4$	$8x10^3$	$8x10^3$	$2x10^4$	$2x10^4$
D	$2x10^4$	$2x10^4$	$2x10^4$	$2x10^4$	$1x10^3$	$2x10^3$	$1x10^3$	$1x10^3$

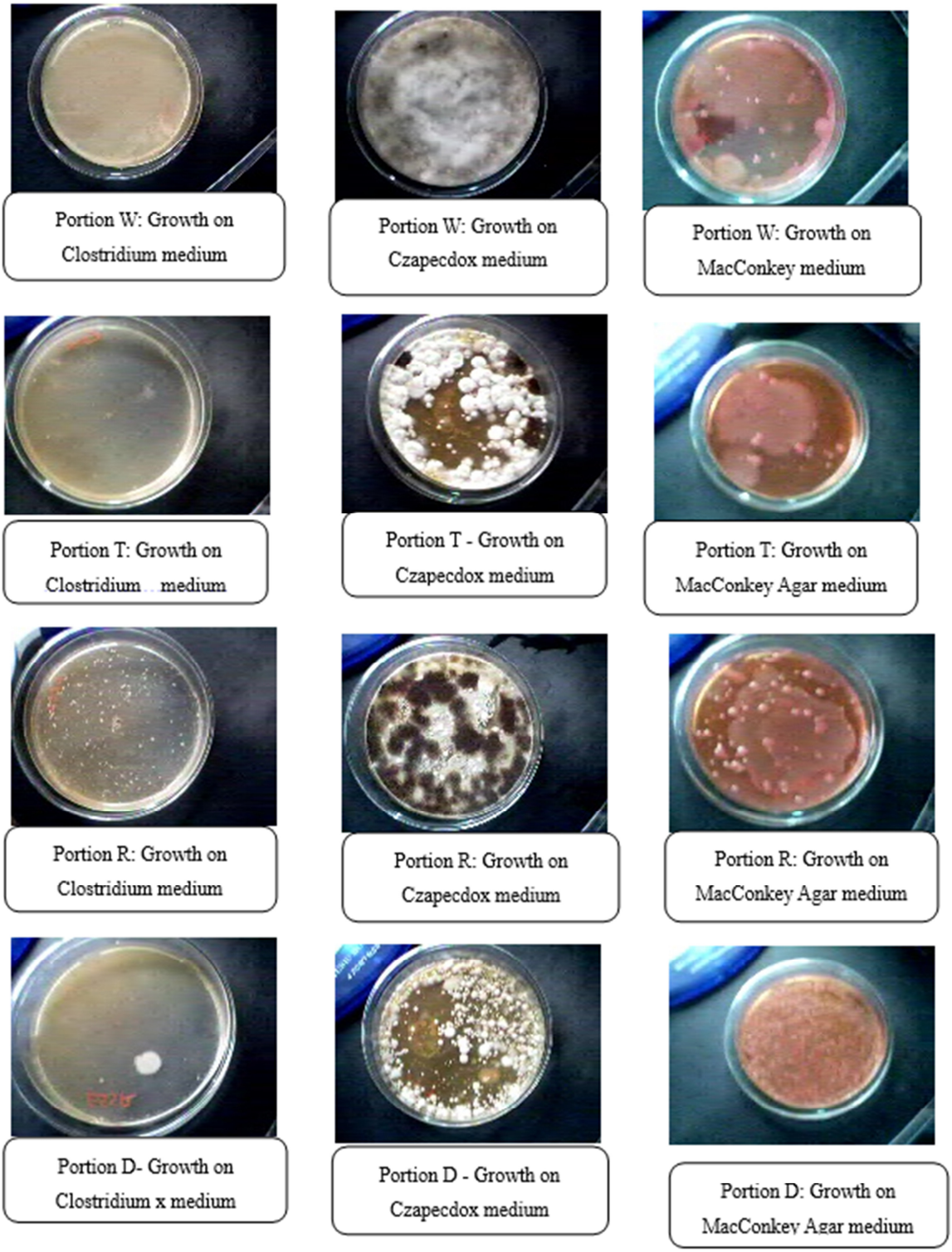
Note:
W = whole portion; T = termite infested region; R = rotted portions and D = discoloured parts.

Table 2: Identification of organisms in the portions of both samples

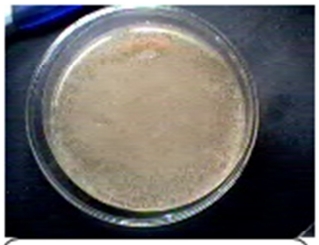
Portion studied	Czapek Dox agar	Microbial load (cfu/g)	*Clostridium* sp	Coliform bacteria (cfu/g)
W	*- Candida sp.* *-Geotricum sp.*	*Bacillus sp* *Pseudomonas sp* *Staphylococcus sp* *Micrococcus sp*	*No clostridium isolated but yeasts grew on medium*	*Proteus sp* *Aeromonas sp*
T	*Penicillium sp* *Aspergillus sp* *Candida sp* *Geotricum sp*	*Bacillus sp* *Pseudomonas sp* *Staphylococcus sp* *Micrococcus sp*	*No clostridium isolated but yeasts grew on medium*	*E. coli* *Proteus* *Aeromonas sp.*
R	*Aspergillus sp* *Candida sp* *Geotricum sp*	*Flavobacterium sp* *Bacillus sp* *Pseudomonas sp* *Staphylococcus sp* *Micrococcus sp*	*No clostridium isolated but yeasts grew on medium*	*E. coli* *Enterobacter sp.* *Proteus sp.* *Aeromonas sp*
D	*Neurospora sp* *Candida sp* *Geotriium sp*	*Bacillus sp* *Pseudomonas sp* *Staphylococcus sp* *Micrococcus sp*	*No clostridium isolated but yeasts grew on medium*	*Enterobacter sp* *Proteus sp*

Note:

Figure 1: Some of the isolates from the samples



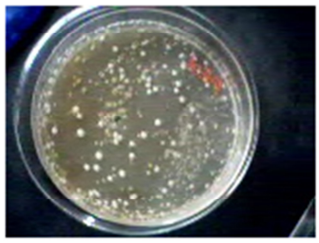
W = whole portion; T = termite infested portion; R = rotted portion and D = discoloured portion.



Portion W: Growth on Nutrient Agar medium

Portion T: Growth on Nutrient Agar medium

Portion R: Growth on Nutrient Agar medium



Portion D: Growth on Nutrient Agar medium

W = whole portion; T = termite infested portion; R = rotted portion and D = discoloured portion.

CONCLUSION

Yams are starchy products and bacterial food poisoning from it is not common unless poorly stored after cooking. Most likely sources of poisoning from yam could be due to mycotoxins when rotten parts are consumed or when wooden mortars/pestles/electric yam pounder have cracked or are poorly cleaned such that fungi grow in them. Inadvertent use of pesticides may also cause food poisoning but this is more common in Nigeria in legumes than in tubers. The above results call for greater priority to be attached to effective management of postharvest storage (for tuberous crops) systems, processing and marketing of foods. Priority areas for action should include:

- proper selection of tubers for food preparation particularly in canteens where many people eat;
- increased awareness by government of the importance of the postharvest sector
- introduction of HACCP system in canteens to avoid any poisoning arising from farms

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