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Investigation of Bacteria and Fungi Associated with Onion (*Allium cepa*) Bulbs Rot Purchased from Markets in Makurdi, Central Nigeria

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Abstract:
Onion (*Allium cepa*) sometimes referred to as the bulb onion and utilized as a vegetable is the most extensively cultivated species of the genus *Allium*. This study evaluated the bacteria and fungi linked to the rot of onion bulbs purchased from markets within the Makurdi metropolis. Samples were analyzed using two dilution factors (10^3 and 10^5) for each sample isolation. All other relevant tests carried out adopted standard microbiological procedures for bacteria and fungi isolation, characterization, and identification. The percentage prevalence of bacterial isolates was; *Escherichia coli* (33.33%), *Pseudomonas* spp (30%), *Staphylococcus* spp (20%), and *Bacillus* spp (16.67%), while fungal isolates were; *Aspergillus* spp (43.33%), *Rhizopus* spp (26.67%), *Mucor* spp (16.67%), and *Saccharomyces cerevisiae* (13.33%). The average bacterial count ranged between 20.8 x 10^4 and 22.4 x 10^6 CFU/ml, while the fungal counts varied between 0.1 x 10^4 and 1.0 x 10^6 CFU/ml. Since these microorganisms are also known to produce toxins harmful to human and animal health, having the right mycological knowledge, storage options, and handling techniques would help to prevent onion bulbs from rotting and subsequently deteriorating, ensuring that they are available to society throughout the year.

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INTRODUCTION

Most Allium species are indigenous to the Northern Hemisphere, mostly Asia. Only a handful are indigenous to Africa, although species from Central and South America thrive in a range of soil types, from wet organic soil to well-drained mineral soil. The majority thrive in sunny areas, although some may grow in woodlands (Vuković *et al.*, 2023). Generally, onions are utilized in processed goods in the form of fresh, preserved, or dehydrated flakes or powders (Savitha *et al.*, 2022).

It is solely cultivated in the Northern region of Nigeria. It is harvested towards the end of March, stored, and distributed as demand requires. The types and sizes of onion bulbs vary; they are often characterized by short stems and falling-off dried scales (Nourbakhsh and Cramer, 2022). Stored onions, frequently exhibit signs of spoilage at every stage of marketing which is not obvious to the purchaser until the bulbs are cut up or further stored. Loss of firmness, watery inner tissues, or moldy layers of leaves is frequent (Petropoulos *et al.*, 2016). Onion rotting is mostly caused by harvesting, processing, and marketing procedures. Storage in tropical countries occurs at ambient temperatures (24–32°C) and varying relative humidity levels based on the place and season. The healthy or damaged bulbs change depending on their original status or the adverse harvesting and storage conditions. It has been observed that large quantities of onions are produced at harvest but after three months half of the produce gets wasted and only a few are taken to the market for sale (Opara, 2003).

Plants are nature's chemical factories; they produce a wide range of compounds, some of which are therapeutic (Ashraf *et al.*, 2020; Iqbal *et al.*, 2019a; Iqbal and Ashraf, 2018; Iqbal and Ashraf, 2019; Shahzad *et al.*, 2017). Onion extract has recently been shown to be effective against microbes and to have a wide range of biological properties, including antibacterial, antioxidant, anti-carcinogenic, anti-mutagenic, anti-asthmatic, immunological modulatory, and probiotic properties (Beigoli *et al.*, 2021; Corzo-Martínez *et al.*, 2007; Sagar and Pareek, 2020; Zhao *et al.*, 2021). Bacteria have a relatively limited role in the market deterioration of foods like onions because most vegetables, such as onions, have an acidic pH value (Alegbeleye *et al.*, 2022). The majority of soft rot of onions during transit or storage is caused by the soft rot coliform bacteria *Erwinia carotovora* and Pseudomonas comparable to *Pseudomonas marginata*. These microorganisms grow on onions in the field before harvesting, following prolonged periods of rain, and when the leaves are drying. Contaminated soil and agricultural residues are the principal source of inoculum for subsequent crops (Barak and Liang, 2008). Insects, irrigation water, and raindrop splash all propagate the germs. It is believed that the deterioration of onions occurs during storage because *Pseudomonas aeruginosa*, which enters into the bulbs only through wounds such as those caused by transplanting, mechanical injuries, or sunscald, contaminates onion bulbs during harvest by moving through wounds caused by topping, resulting in soft rot (Oricha, 2019).

Due to the health benefits, high rate of consumption, and economic importance of onions especially in Nigeria, it has become imperative to study the spoilage of this essential food and food ingredient and report the microbes associated with the rot of onions in Nigeria.

MATERIALS AND METHODS

Sample collection

Samples of onion were acquired from onion vendors at High Level, Wurukum, and North Bank Markets in Makurdi. The samples were promptly wrapped in sterile, distinctive nylon to avoid cross-contamination from different sampling areas and then transported to the Departmental laboratory of the Federal University of Agriculture, Makurdi for analysis.

Sterilization of Materials

All glassware used in the course of this work, such as measuring cylinders, pipettes, conical flasks, beakers, test tubes, Petri dishes, laboratory mortar, and pestle, were thoroughly washed and sterilized in a hot air oven at 160 – 200°C for two hours. Wire loops and inoculating needles were sterilized by flaming.

Microbiological analysis

Serial dilution up to five folds (10^{-5}) in test tubes was carried out on samples, using a sterile pipette in each case. The serial dilution was done after using the rinsing method (that is, rinsing the onion in distilled water). It was carried out with 1ml of the various stock solutions from the different samples in 9ml of distilled water into 10^{-1}, 10^{-2}, 10^{-3}, $10^{-4,}$ and 10^{-5} respectively.

Media Preparation

All media used for inoculation and isolation of organisms were prepared aseptically following the manufacturer's recommendations. Media was dissolved in conical flasks containing distilled water and corked using cotton wool and aluminum foil. After which, they were sterilized in an autoclave at 121°C for 15 minutes and allowed to cool to about 45°C before pouring into Petri dishes (Bello and Echevarría, 2022; Cheesbrough, 2018; Odo *et al.*, 2023).

Inoculation of Samples

After the serial dilution, 1ml of each diluent from dilutions 10^{-3} and 10^{-5} was inoculated into duplicate sterile Petri dishes using a sterile pipette. Thereafter, the prepared media (Nutrient agar and Sabouraud dextrose agar SDA) were poured into the inoculated Petri dishes spread by swirling; and then allowed to solidify. The plates for bacterial growth were incubated at 37°C for 24 hours, while plates for fungal isolates were incubated at room temperature (25°C) for 72 hours (Kalim *et al.*, 2016; Khalid *et al.*, 2016; Yunus *et al.*, 2016).

For the Sabouraud dextrose agar (SDA), 0.5ml streptomycin was added to the SDA medium before dispensing it. This was done to suppress or eliminate the growth of any bacteria that might tend to grow on the medium which was meant for the growth and isolation of fungi (Cheesbrough, 2018; Punja *et al.*, 2023).

Colony Count

This was performed on the various cultures with Nutrient Agar and Sabouraud Dextrose Agar, after incubation. Following the proper incubation, discrete colonies that appeared on the plate were counted and noted. On nutrient agar, separate colonies were counted to determine the overall number of bacteria while fungal counts were obtained on the SDA (Esmail *et al.*, 2020; Iqbal *et al.*, 2015).

Microorganisms present in the original sample were calculated thus:

$$\text{CFU/ml} = \frac{\text{Number of Colonies X Dilution factor}}{\text{Volume of Sample Plated}},$$

Where the volume of the sample plated is = 1 ml.

Isolation of Pure Culture and Identification of Bacterial Isolates

For further identification, one discrete colony isolate was taken from each culture plate and further subculture was established on nutrient agar and eosin methylene blue agar, and it was incubated for 24 hours at 37°C. Characterization and identification of the isolates was done based on their cultural, morphological, and biochemical characteristics using standard procedures described in previous studies (Hussain *et al.*, 2016; Iqbal *et al.*, 2016; Iqbal and Ashraf, 2020; Kalim *et al.*, 2016; Mohammad *et al.*, 2021).

Fungal Identification

A scalpel and pin were used to delicately remove a little amount of the fungal culture that was then placed on a clean glass slide, stained with lacto phenol cotton blue reagent, and viewed under the low power (x10) and high power (x40) objectives. The isolates were identified by taking notice of the hyphal

structure, spore, shape, and arrangement. By closely examining the cultures and analyzing their look and color, the cultures were initially identified (Esmail *et al.*, 2020).

RESULTS

Frequency of Isolated Bacteria and Fungi species in the Onion Samples.

Data from Table 1 shows the frequency of the bacteria isolated from the onion samples. The incidence of bacterial isolates was *E. coli* 10 (33.33%), followed by *Bacillus* spp. 5 (16.67%), *Pseudomonas* spp. 9 (30%), and *Staphylococcus* spp. 6 (20%). All the isolates were isolated from at least one of the various sample locations.

Table 2 shows the frequency of the fungi isolated from the onion samples. *Aspergillus* spp had the highest frequency of 13 (43.33%), followed by *Rhizopus* spp 8 (26.67%), *Mucor* spp. 5 (16.67%) and *Saccharomyces* spp. 4 (13.33%). All the isolates were isolated from at least one of the various sample locations except for *Mucor* spp and *Saccharomyces cerevisiae* which were not isolated from High Level and Wurukum, respectively.

Table 1. Frequency of Isolated Bacteria species from the Onion Samples.

Sample Location	Number of samples	*Escherichia coli*	*Pseudomonas* spp	*Staphylococcus* spp	*Bacillus* spp
High Level	10	2	4	2	2
North Bank	10	5	2	2	1
Wurukum	10	3	3	2	2
Total (%)	30 (100)	10 (33.33)	9 (30)	6 (20)	5 (16.67)

Table 2. Frequency of Fungi species in Onion Samples.

Sample Location	Number of samples	*Aspergillus spp*	*Rhizopus spp*	*Mucor spp*	*Saccharomyces cerevisiae*
High Level	10	6	2	0	2
North Bank	10	3	4	1	2
Wurukum	10	4	2	4	0
Total (%)	30 (100)	13 (43.33)	8 (26.67)	5(16.67)	4 (13.33)

Plate Count on Nutrient Agar and Sabouraud Dextrose Agar from Various Locations

The plate count on Nutrient and Sabouraud dextrose Agar from the various locations within the High-Level Market is presented in Table 3. The first three samples (1, 2, and 3) for each location analyzed under the dilution factors are the samples collected from store sellers, while the remaining samples analyzed (4 and 5) for the various locations were those samples collected from hawkers. The plate count for samples purchased from store sellers was 21.7×10^6 CFU/g on Nutrient Agar; this was higher than those purchased from hawkers 1.0×10^6 CFU/g on Sabouraud dextrose Agar.

Table 3. Plate count on nutrient agar and sabouraud dextrose agar from the various locations within the High level.

Sample Location	Seller	Dilution Factor	Nutrient Agar (CFU/ml)	Sabouraud Dextrose Agar (CFU/ml)
High Level 1		10^3	20.8×10^4	1.6×10^4
	Store	10^5	15.7×10^6	0.8×10^6
High Level 2	Sellers	10^3	23.8×10^4	0.1×10^4
		10^5	13.5×10^6	0.1×10^6
High Level 3		10^3	30.2×10^4	0.3×10^4
		10^5	21.7×10^6	0.3×10^6
High Level 4		10^3	31.8×10^4	0.4×10^4
	Hawkers	10^5	21.0×10^6	0.2×10^6
High Level 5		10^3	25.0×10^4	0.4×10^4
		10^5	20.0×10^6	1.0×10^6

Tables 4 and 5 show the plate count on both Nutrient and Sabouraud dextrose agar from the various locations within North Bank and Wurukum Markets respectively. Onion bulbs purchased from store sellers showed a plate count of 21.6×10^6 CFU/g on Nutrient agar. Those purchased from hawkers were 0.8×10^6 CFU/g on Sabouraud dextrose agar (Table 4). The plate count on Nutrient agar and Sabouraud dextrose agar from Wurukum Market reflected onions purchased from hawkers to be 22.4×10^6 CFU/g and 0.6×10^6 CFU/g on Nutrient and Sabouraud Dextrose agar respectively (Table 5).

Table 4. Plate Count on Nutrient Agar and Sabouraud Dextrose Agar from the Various Locations within North Bank.

Sample Location	Seller	Dilution Factor	Nutrient Agar (CFU/ml)	Sabouraud Dextrose Agar (CFU/ml)
North Bank 1		10^3	29.2×10^4	0.3×10^4
		10^5	17.9×10^6	0.2×10^6
North Bank 2	Store	10^3	29.4×10^4	1.2×10^4
	Sellers	10^5	15.6×10^6	0.8×10^6
North Bank 3		10^3	28.8×10^4	1.4×10^4
		10^5	21.6×10^6	0.8×10^6
North Bank 4		10^3	30.2×10^4	0.9×10^4
	Hawkers	10^5	20.8×10^6	0.7×10^6
North Bank 5		10^3	26.8×10^4	1.0×10^4
		10^5	19.1×10^6	0.6×10^6

Table 5. Plate Count on Nutrient Agar and Sabouraud Dextrose Agar from the Various Locations within Wurukum.

Sample Location	Seller	Dilution Factor	Nutrient Agar (CFU/ml)	Sabouraud Dextrose Agar (CFU/ml)
Wurukum 1		10^3	27.6×10^4	1.1×10^4
		10^5	11.7×10^6	0.1×10^6
Wurukum 2	Store	10^3	30.2×10^4	0.5×10^4
	sellers	10^5	12.4×10^6	0.2×10^6
Wurukum 3		10^3	30.0×10^4	1.1×10^4
		10^5	21.6×10^6	0.3×10^6
Wurukum 4		10^3	29.4×10^4	0.8×10^4
	Hawkers	10^5	22.4×10^6	0.6×10^6
Wurukum 5		10^3	29.9×10^4	0.7×10^4
		10^5	18.6×10^6	0.3×10^6

DISCUSSION

This study presented the isolation of bacteria and fungi from the onion samples. Raw onions can degrade for a variety of reasons, including physical conditions, the operations of their enzymes, bacteria, or a combination of such factors. Mechanical damage brought on by the acts of animals, birds, or insects, or by careless handling, may be predisposed to enhance enzymatic activity or the entry and proliferation of microbes (Buell *et al.*, 2003). Among the bacterial isolates, *Escherichia coli* showed the highest occurrence (33.33%). Our results agreed with a previous study that reported a higher incidence of *E. coli* from onions with brown rot symptoms (Kelechi and Joseph, 2020). Another study reported the isolation of three bacterial species causing onion spoilage which comprise *Staphylococcus* spp, *Bacillus* spp, and *Erwinia* spp, with *Staphylococcus* spp having the highest incidence (Roopa *et al.*, 2014). The results may be influenced by environmental factors and sample location. The presence of *Staphylococcus* spp in the samples could be attributed to the fact that healthy humans are healthy carriers of *S. aureus* (Okigbo *et al.*, 2009). *Staphylococcus* spp, are known to cause diseases such as septicemia, food poisoning, abscesses, and carbuncles in humans (Al-Khawlany *et al.*, 2021; Iqbal and Ashraf, 2021; Pollitt *et al.*, 2018; Saleem *et al.*, 2020; Tong *et al.*, 2015).

These microorganisms can be used as indicators of inadequate product handling. *E. coli* is used to assess the sanitary quality of food products; hence, its presence in onions in high proportion is undesirable and a challenge. The incidence of *E. coli* shows that the contaminants may be from warm-blooded animal fecal origins (Aslam *et al.*, 2021). This might be due to possible contaminations of fresh onion during irrigation or harvest. The enterotoxigenic form causes travelers' diarrhea and usually spreads through contaminated water and food (Qadir, 2019). Thus, the issue of cross-contamination of the product is quite certain.

The portion of the crop utilized for food that has previously been harmed by plant diseases may no longer be appropriate for eating or it may have created a space for saprophytes to develop and cause spoiling. Contact with spoiled onions may result in the transmission of microbes that cause them to deteriorate and increase their wastage. When harvested, transported, stored, and marketed, unfavorable environmental conditions may encourage rotting (Gorrepati *et al.*, 2017; Gupta *et al.*, 1986; Schroeder *et al.*, 2012). To maintain the necessary temperatures and humidity levels within the store without causing water to condense on the surface, ventilation must be performed properly (Opara, 2003).

Fungi have been linked as contaminants of agricultural land, sand, and water resources (Ashraf and Iqbal, 2022; Echevarría, 2022; Echevarría and Bello, 2023; Iqbal and Ashraf, 2023; Onuegbu and Dimkpa, 2010). Both people and animals have been known to contract infections from these fungi. They might originate from the handlers, the soil, the water, or even the air (Echevarría, 2019; Echevarría and Iqbal, 2021; Iqbal *et al.*, 2019b).

From this study, the *Aspergillus spp.* reported the highest occurrence (43.33%) consistent with previous studies (Samuel and Ifeanyi, 2015). Our results disagree with a study that described *Rhizopus spp* as the most prevalent fungi in their research (Chukwuka *et al.*, 2010). Results from other researchers indicated that the most destructive pathogens in storage are *Aspergillus niger* (black mold), *Aspergillus* spp (Aspergillus rot), *A. niger* and *A. flavus* infect bulbs at high temperatures with high relative humidity (El-Nagerabi and Ahmed, 2003; Mousavi *et al.*, 2016; Toma, 2021).

Aspergillus niger and *Rhizopus stolonifer* were also recovered from decaying onion bulbs sold at five different Sokoto, Nigerian marketplaces (Shehu and Muhammad, 2011). *A. niger* was identified as the culprit behind the black mold rot of onions. One frequent reason for onion loss during storage is bulb rots mainly caused by fungus (Narayana *et al.*, 2007). *A. niger* is

associated with hot climate-produced onion seeds, and its transfer from soil and naturally infected seeds to onion seedlings results in 30-80% loss or rotting of onion bulbs (Samuel and Ifeanyi, 2015).

The environmental factors, onion handling and processing practices, onion storage conditions, onion handler fungal loads, and onion bulb quality all contribute to the occurrence of these fungi in onion bulbs (Yeshiwas *et al.*, 2023). Since fungal infections have been shown to enhance the likelihood of contamination by mycotoxins, which pose significant risks to both human and animal health (Iqbal *et al.*, 2021). Therefore, it poses a threat to public health when these fungi are found in large quantities in onion bulbs.

CONCLUSION

The bacteria isolated from the various markets were *Escherichia coli, Pseudomonas spp, Staphylococcus spp, Bacillus spp,* and the fungi isolated were *Aspergillus spp, Rhizopus spp, Mucor spp, and Saccharomyces spp.* rotten onion bulbs can serve as a reservoir for microbes. These pathogenic organisms reported can pose health issues to consumers. To safeguard the onion bulbs from these microorganisms' assaults and reduce waste from degeneration and unacceptability, good storage facilities should be set up.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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