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Study of Microorganisms Associated with Spoilage of Onions Sold in Mbiama Market, Rivers State, South South Nigeria

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Abstract

Onions are a staple vegetable in many Nigerian households, and their spoilage can contribute to significant economic losses and food insecurity. This study investigated the microorganisms associated with the spoilage of onions sold in Mbiama Tuesday Market, Rivers State, South-South Nigeria, and examined their potential implications for public health. A total of 100 onion samples were collected, and the associated microorganisms were isolated, identified, and characterized using standard bacteriological and mycological techniques, including biochemical testing and antibiotic sensitivity assays. The results showed that the onions were contaminated with a variety of bacteria—*Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp., and *Escherichia coli*—and fungi, including *Mucor*, *Aspergillus niger*, yeast, and *Penicillium* spp., all of which were capable of causing soft rot, discoloration, and decay. Among the bacterial isolates, *E. coli* was the most frequent (23.5%), whereas *Pseudomonas* spp. was the least frequent (9.8%); among the fungi, *A. niger* was the most frequent (13.7%) and *Mucor* the least frequent (5.9%). Bacterial colony counts ranged from 5.2×10^5 CFU/g (*E. coli*) to 5.6×10^5 CFU/g (*Pseudomonas* spp.), while fungal counts ranged from 1.4×10^5 CFU/g (yeast) to 1.58×10^5 CFU/g (*A. niger*). Biochemical tests revealed that *Bacillus* spp. and *Staphylococcus* spp. were

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catalase and coagulase positive, whereas *Pseudomonas* spp. and *E. coli* were coagulase negative. Antibiotic sensitivity testing showed that *Staphylococcus* spp. was resistant to the tested antibiotics, while *Bacillus* spp., *E. coli*, and *Pseudomonas* spp. remained sensitive. The study concludes that onions sold in Mbiama Market are contaminated with diverse spoilage-causing bacteria and fungi, posing a potential risk to public health. These findings underscore the need for proper handling, storage, and preservation practices, such as maintaining cool, dry storage conditions and ensuring hand hygiene among vendors and consumers as well as further research to develop effective strategies for controlling microbial spoilage of onions in Nigeria.

Keywords: Onion Spoilage; Microbial Contamination; Food Safety; Public Health; Rivers State Nigeria.

Introduction

Onions (Allium cepa) are a staple vegetable in many Nigerian households, and their spoilage can lead to significant economic losses and food insecurity (Dimkpa and Onuegbu, 2010). Onions are susceptible to spoilage by various microorganisms, including bacteria and fungi, which can cause soft rot, discoloration, and decay (Baiyewu et al., 2007). Several studies have investigated the microorganisms associated with the spoilage of onions in different parts of the world (Narayana et al., 2007). In Nigeria, studies have reported the presence of fungi such as *Aspergillus niger, Penicillium* spp., and *Fusarium* spp. on spoiled onions (Adebayo et al., 2015; Eke et al., 2017). Similarly, bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* have been isolated from spoiled onions in Nigeria (Oguntoyinbo et al., 2013; Okwu et al., 2015). In other parts of the world, studies have reported the presence of microorganisms such as *Erwinia carotovora, Pseudomonas cepacia*, and *Aspergillus flavus* on spoiled onions (Lacy et al., 1992; Snowdon, 1991). These microorganisms can produce enzymes that break down the cell walls of onions, leading to soft rot and decay. The spoilage of onions can be influenced by various factors, including handling and storage conditions, temperature, humidity, and the presence of wounds or injuries on the onion surface (Barkai-Golan, 2001). In Nigeria, onions are often stored in open markets or in poorly ventilated stores, which can contribute to the growth of microorganisms and spoilage (Adebayo et al., 2015). Onion is known for being a good natural source of flavonoids mainly represented by the flavonols - quercetin and kaempferol, which are present as their glycosides (Roopa et al., 2014). Protection of food

from pathogens and spoilage organisms has been traditionally achieved by chemical methods, but during recent years there has been an increase in consumer interest in developing Onion which contain a low level or are free of chemical preservatives (Shahedur et al., 2011). The emergence of pathogens which are resistant to classical preservatives has also created an urgent necessity to find alternative antimicrobial agents (Shehu and Mohammad, 2011). In consequence, the food industry is interested in developing natural components for the partial replacement of synthetic antimicrobials (Shehu and Mohammad 2014). Onions can be considered as a good source of natural additives to retard food deterioration. Numerous benefits have been attributed to onions including prevention of cancer and cardiovascular disorders, reduction in the blood levels of cholesterol, reduction in osteoporosis (Snowdon, 1991). Reduction in stomach ulcers, inhibition of the proliferation of cultured ovarian, breast and colon cancer cells, inhibition of platelets-mediated thrombosis, prevention of inflammatory processes associated with asthma, treatment of fever, common cold, cough, sore throat and its use as an antimicrobial agent (Tyson and Fulerton 2004). Onions are packed locally in baskets and jute bags. These packaging materials come from palm, bamboo and fibrous jute trees. However, these materials provide no barriers of dust, and can easily be crushed which might lead to damage of the onions. During storage, some losses occur due to sprouting, drying and rotting. Bulb rots are a common cause of onion loss during storage. They are caused by microorganisms particularly fungi. The black mould disease caused by *Aspergillus niger* is a limiting factor in onion production worldwide. *Aspergillus niger* has been reported to survive between onion crops as a soil saprophyte in or on bulbs in field or storage and is ubiquitous in nature (Oyeleke and Manga, 2008). The fungus invades bulbs of onions in field or storage whenever they find injured tissues by producing various enzymes or toxins (Narayana et al., 2007). Association of *Aspergillus niger* with onion seeds produced in hot climates and their transmission from soil and naturally-contaminated seeds to onion seedlings cause 30-80% loss or spoilage of onion bulbs (Narayana et al., 2007). Mbiama Market in Rivers State, Nigeria, is a major hub for onion trade, and the onions sold in this market are often handled and stored under poor conditions, making them susceptible to microbial contamination. Despite the importance of onions in the Nigerian diet, there is a dearth of information on the microorganisms associated with their spoilage in this region. This study aimed to investigate the microorganisms associated with the spoilage of onions sold in Mbiama Market, Rivers State, Nigeria. The investigation of microorganisms

associated with the spoilage of onions is important for developing effective methods for controlling spoilage and ensuring food safety. This study aimed to investigate the microorganisms associated with the spoilage of onions sold in Mbiama Market, Rivers State, Nigeria.

Materials and Methods

The study was conducted in Mbiama, Rivers State. It has an estimated area of 527km2 (203sqkm) and a population of 0ver 500,000 as at 2006 census (FRN, 2006). The population and activities in the area have increased in the last years which may be due to the establishment of the new Federal University in a neighbouring State of Bayelsa. The area is bounded by Ahoda and Yenegoa.

Sample collection

The onion bulbs were randomly selected from the markets and packaged in sterile polythene bags and transported to the Microbiology Laboratory of the Department of Microbiology Federal University Otuoke for analysis.

Media Preparation

The media used for the isolation and characterization of microorganisms were prepared according to the manufacturer's instructions. The media used included:

- Sabouraud Dextrose Agar (SDA) for fungal isolation
- Nutrient Agar (NA) for bacterial isolation
- Nutrient Broth (NB) for enrichment
- MacConkey Agar (MA) for Gram-negative bacterial isolation
- Eosin Methylene Blue (EMB) Agar for Gram-negative bacterial isolation (Cheesbrough, 2006).

Isolation of Bacteria and Fungi

The surface of the spoiled onion bulb was wiped with 70% alcohol. A sterile razor blade was used to cut about 1g of the spoiled onion tissue, which was then transferred to a sterile mortar and pestle. Sterile distilled water was added, and the mixture was crushed.

One milliliter (1ml) of the sample was added to 9ml of sterile Nutrient Broth in a Universal bottle, and the mixture was allowed to stand for 15 minutes.

Serial dilutions were performed by transferring 1ml of the sample to 9ml of sterile distilled water, and this process was repeated up to 10^(-5) dilution.

Inoculation and Incubation

For bacterial isolation, 0.5ml of the aliquot from the serial dilution was inoculated onto NA, MA, and EMB Agar plates, which were then incubated at 37°C for 24 hours.

For fungal isolation, SDA plates were prepared with Chloramphenicol (antibiotic) to prevent bacterial growth. The same procedure as for bacterial isolation was followed, and the plates were incubated at room temperature for 5-7 days.

Microbial Colony Count

Bacterial and fungal colonies were counted on each plate to determine the number of colonies. The count was obtained by multiplying the number of colonies by the dilution factor.

Macroscopy and Colonial Identification

The colonies were examined for their morphological characteristics, including size, shape, color, elevation, and texture. The results were recorded.

Identification of Bacteria and Fungi

Bacterial isolates were identified based on their Gram reaction, biochemical reactions, and cultural characteristics.

- Gram staining was performed to differentiate Gram-positive from Gram-negative bacteria.

- Biochemical tests, such as catalase, oxidase, and sugar fermentation, were performed to further identify the isolates.

Fungal isolates were identified based on their microscopic morphology, including the shape and size of conidia, hyphae, and other structures.

- Lactophenol Cotton Blue (LPCB) staining was used to examine the fungal morphology.

Antibacterial Sensitivity Testing

The antibacterial sensitivity test was performed to determine the susceptibility of bacterial isolates to various antibiotics. A standardized disk diffusion method was used, following the Standards Microbiological techniques.

Antibiotic Sensitivity Testing

The following antibiotics were tested against the bacterial isolates:

- Gentamycin (10µg)
- Streptomycin (10µg)
- Septrin (30µg)
- Chloramphenicol (30µg)
- Ciproflaxin (10µg)
- Ampiclox (30µg)
- Erythromycin (10µg)
- Ampicillin (30µg)

Procedure

1. Bacterial isolates were grown in Mueller-Hinton broth at 37°C for 18 hours with shaking.

2. The cultures were diluted in optical density of 0.1 (0.5 McFarland standard) and stored at 4°C.

3. The antibiotic discs were gently placed on the inoculated Mueller-Hinton agar plates using sterile forceps.

4. The plates were incubated at 37°C for 24 hours.

5. The zones of inhibition were measured and interpreted according to the standard microbiological guidelines.

Interpretation of Results

The results were classified as:

- Sensitive (S): The bacterial isolate is susceptible to the antibiotic.

- Resistant (R): The bacterial isolate is resistant to the antibiotic.

Statistical Analysis

The frequency of occurrence of bacteria and fungi isolated from spoiled onions was determined using simple percentage calculations.

Results

The isolated bacteria from fresh onions are *Bacillus* spp, *Staphylococcus* spp, *Pseudomonas* spp and *Escherichia coli* as shown on table 1. Isolated fungi are Mucor, *Aspergillus niger*, Yeast and *Pencillium* spp was isolated from the onions as shown on table 2. Table 3: Frequency of occurrence of Bacteria and Fungi Isolated from the Spoilt Onions sold in Mbiama Tuesday market. The biochemical reaction test for bacteria isolates is shown in table 4. Table 5, shows identification and characterization of fungi isolated. Antibiotics sensitivity pattern of bacterial isolates obtained from the spoilt Onions antibiotics used on bacterial isolates as show in table 6.

Table 1: Total Bacteria Colony Count from Samples Collected from Mbiama Market.

S/N	ISOLATES	COLONY COUNT
1	Bacillus spp	5.0×10^5
2	Pseudomonas spp	5.2×10^5
3	Staphylococcus spp	5.6×10^5
4	Escherichia coli	2.2×10^5

Table 2: Total Fungi Colony Count from Samples Collected from Mbiama Market.

S/N	ISOLATES	COLONY COUNT
1	Mucor	1.6 X 10^5
2	Yeast	1.4 X 10^5
3	Aspergillus niger	1.58 X 10^5
4	Penicillium spp	1.52 X 10^5

Table 3: Frequency of occurrence of Bacteria and Fungi Isolated from the Spoilt Onions sold in Mbiama Tuesday market.

Organisms	Number of Occurrence	Percentage (%) of Occurrence
Bacillus spp	7	13.7
Pseudomonas spp	5	9.8
Staphylococcus spp	8	15.6
Escherichia coli	12	23.5
Mucor	5	11.7
Yeast	4	7.8
Aspergillus spp	7	13.7
Penicillium spp	3	5.9
Total	51	100

Table 4: Biochemical Reaction Test for Bacteria Isolates.

Organisms	Gram SRXN	CAT	MOT	MR	IND	CIT	COA
Bacillus spp	+SR	+	-	-	-	-	+
Staphylococcus spp	+C	+	-	-	-	-	+
Pseudomonas spp	-LR	+	+	-	-	+	-
Escherichia coli	-R	+	+	+	+	-	-

+C: Gram Positive Cocci; -: Negative; +: Positive; -R: Gram Negative Rod;

+R: Gram Positive Rod; CAT: Catalase; MO: Motility; IND: Indole; CIT: Citrate; COA: Coagulase; Gram SRXN: Gram Stain Reaction; MR: Methyl Red.

Table 5: Identification and characterization of fungi isolated

Organisms	Microscopic	Macroscopic
Mucor	Thick non-septate hyphae with dark sporangiospore	White, heavy, crocheted fluffy growth covering entire plate
Aspergillus spp	Thick septate hyphae with conidiaborn in chains from the sterigmata	Black colonies with white edges
Yeast	Oval in shape with very short Multidimensional budding	Small Soft or Whitish colonies
Penicillium spp	Brush-like conidiophores typically branched	Greyish- green, velvet-like colonies

Table 6: Antibiotics Sensitivity Pattern of Bacterial Isolates obtained from the spoilt Onions Antibiotics used on bacterial isolates.

ISOLATES	CH	CN	AM	PEF	OFX	S	AU	SXT	SP	CPX
Bacillus spp	**10**	**20**	**24**	**23**	**8**	**24**	**11**	**22**	**6**	**30**
Pseudomonas spp	4	6	2	4	5	2	1	10	6	2
Staphylococcus spp	20	30	28	37	30	30	32	27	28	38
Escherichia coli	22	21	24	23	36	23	21	10	28	36

Greater than 19mm: Susceptible; Less than 13mm: Resistance; CH: Chloramphenicol; SXT: Septrin; Am: Amoxicillin; CN: Gentamicin; S: Streptomycin; OFX: Tarivid; Au: Augmentin; AM: Amoxicillin; PEF: Pefloxacin; SP: Sparfloxacine.

Discussion

The present study isolated several microorganisms from spoiled onion bulbs, including *Staphylococcus* spp, *Bacillus* spp, *Pseudomonas* spp, *Escherichia coli*, Mucor, Yeast, and *Aspergillus niger* and *penicillium* spp. The fungal isolates were identified on Sabouraud Dextrose Agar (SDA), while the bacterial isolates were identified on Nutrient Agar (NA). The isolation of these microorganisms is consistent with previous studies. For example, Baiyewu et al. (2015) isolated similar fungi from garlic and pawpaw fruits in Nigeria. Similarly, Shehu and Muhammad (2014) reported a high frequency of occurrence of

Aspergillus niger in onion bulbs. The presence of these fungi in onion bulbs can be attributed to environmental conditions, handling and processing, storage, and the quality of the onion bulbs (Akinyele and Abiodun, 2014). The isolated fungi, including *Aspergillus niger*, *Penicillium* spp, Mucor, and Yeast, are known to cause diseases in humans and animals, such as Ringworm and Aspergillosis (Samuel and Ifeanyi, 2013). These fungi can also produce mycotoxins, which are hazardous to health. The bacterial isolates, including *Staphylococcus* spp, *Bacillus* spp, *Pseudomonas* spp, and *Escherichia coli*, are also of public health concern. *Bacillus* spp is known to cause food poisoning, while *Staphylococcus* spp is a common cause of skin infections (Liao and Wells, 2015). The antibiotic sensitivity test results showed that some of the isolates, including *Pseudomonas aeruginosa*, *Bacillus* spp, and *Escherichia coli*, were sensitive to Chloramphenicol, Septrin, Amoxicillin, Gentamicin, Streptomycin, Tarivid, Augmentin, Pefloxacin, Sparfloxacine, and Ciprofloxacin. However, *Staphylococcus* spp was resistant to these antibiotics.

Conclusion

The results of this study indicate that spoiled onion bulbs are a reservoir of both fungal and bacterial microorganisms that can cause spoilage and pose a risk to human health. Contamination can occur due to exposure to poor environmental conditions, such as humidity and temperature. Proper handling, storage, and processing of onions are necessary to prevent spoilage and reduce the risk of foodborne illness.

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