



Deterioration Profile of Postharvest Onion (*Allium cepa* L.) Bulbs Induced by Potential Pathogenic Microorganisms

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Author's contributions

This work was carried out in collaboration among all authors. Author NT developed the concept and designed the study. Authors IOM, RKO and OBD conducted the microbial analysis of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2020/v5i230130

Editor(s):

(1) Dr. Khadiga Ahmed Ismail Eltris, Ain Shams University, Cairo, Egypt.

Reviewers:

(1) Saada Mohamed Nour Kheiry, University of Bahri, Sudan.

(2) M. A. Khan, ICAR-Indian Agricultural Research Institute, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/61173>

Original Research Article

Received 06 July 2020
Accepted 12 September 2020
Published 24 September 2020

ABSTRACT

Onion (*Allium cepa* L.) is a highly nutritive vegetable with about 2 million metric tons grown annually in Nigeria, but the majority is lost to postharvest spoilage, especially through microbial infection. In this study, we identified bacteria and fungi associated with postharvest spoilage in onion bulbs and determined the pathogenicity of the bacterial isolates. Two weeks stored onion bulbs were purchased at a market in Ile-Ife, rinsed in 5% HOCL and aseptically cut into seven sections each. The fourteen sections obtained were swabbed daily with sterile cotton-tipped applicators for seven days. The swabs were streaked onto the surface of Nutrient Agar (NA) and selective/differential media plates for the isolation of bacteria, and Potato Dextrose Agar (PDA) plates for the cultivation of fungi. The bacterial plates were incubated at 37°C for 24 hours, while the fungal plates were incubated at 25°C for 5 days. The isolates were identified based on standard microbiological methods. Pathogenicity tests of the bacterial isolates from each of the genera was carried out by re-inoculation on the inner tissues of fresh onion bulbs that have been cleaned with 1% NaOCL, an uninoculated onion bulb served as the control. Thirty-five (35) bacterial isolates belonging to four different genera were identified, which included; 11 (31.4%) *Staphylococcus* spp., 9 (25.7%) *Micrococcus* spp., 8 (22.9%) *Bacillus* spp. and 7 (20%)

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Flavobacterium spp. Seven (7) fungal isolates were identified which included; 5 (71.4%) *Aspergillus fumigatus*, 1 (14.3%) *Gibellula suffulta* and 1 (14.3%) *Hirsutella saussueri*. Pathogenicity tests revealed that all the bacterial isolates were able to cause rot in onion in comparison with the control which had no observable rot; *Flavobacterium* spp. (28 mm) was the most pathogenic, while *Micrococcus* spp. was the least pathogenic (14 mm) based on the diameter of rot formation within seven days. These findings revealed that spoilage microorganisms can cause onion rot, hence, onions already showing contamination symptoms should be separated from fresh ones to avoid cross-contamination, while adequate care should be taken before consumption of onion to avoid foodborne illnesses and diseases.

Keywords: Onion; postharvest spoilage; food safety; bacteria; pathogenicity test; fungi.

1. INTRODUCTION

Vegetables are the edible components of plants like leaves, bulbs, seeds, stalks that could be useful in making soup or serve as an integral part of the main meal [1]. These vegetables are valued because they are made up of high vitamin and mineral contents that are necessary for the maintenance of health by preventing diseases and ensuring that the body functions properly [2]. Onion (*Allium cepa* L.) is a vegetable that is widely grown with an annual world production of about 88.5 million metric tons and about 2 million metric tons in Nigeria [3]. Onion not only contains vitamins and minerals but phytonutrients and polyphenols that make it useful in the prevention of diabetes, cancer and high blood pressure [4].

Onion is known to be highly nutritive but it requires strict maintenance and appropriate storage conditions to maintain its nutritive contents [5]. The shelf life of onion is reduced by contamination with pathogenic bacteria and fungi that cause postharvest diseases [6,7]. Besides, about 20-30% postharvest loss of onion is attributed not only to microbial infection but also to mechanical damage during the process of transportation [8,9]. Consuming contaminated fruits and vegetables has been established as one of the main sources of diverse diseases in humans [2,10,11,12,13]. Some of the bacteria that have been isolated from onion and other vegetables include; *Klebsiella* sp., *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter* sp., *Pseudomonas* sp., *Flavobacterium* sp. and *Escherichia coli* [14,15]. *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifera*, *Aspergillus fumigatus*, *Fusarium* sp., are examples of fungi that have been isolated from onion and other vegetables [16,14]. The sources of these pathogenic bacteria and fungi that cause food poisoning have been reported to include fertilizer, soil, irrigation water, air, insects,

livestock/wildlife, farmworkers, harvesting and transportation equipment [17,18,19].

It has been reported that several microbial pathogens can co-exist and co-express their virulence to increase the severity of diseases in onion tissues [20]. This suggests that the interaction between plants and pathogens in natural environments is beyond the scope of the conventional one pathogen causing one disease, but an inter-play of multiple pathogens [21]. The management of onion bulb rot diseases in Nigeria needs an adequate understanding of the occurrence and diversity of pathogenic microbial community associated with onion spoilage. Although, this occurrence and diversity may vary with location, soil properties, climatic factors, and management practices. This study isolated and identified different microorganisms associated with the spoilage of onion in storage, and examined the ability of the isolated bacteria to cause rot formation in fresh onion bulbs after re-inoculation, giving a fantastic insight into the postharvest management of onion bulb rot diseases in Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Two deteriorating bulbs of a single variety of purple-red skinned onion cultivar Goalmi (*Allium cepa* L.) considered to have undergone the same management practices, storage conditions and of the same origin (place of cultivation) that have been stored in a basket for two weeks were purchased at the central market, Obafemi Awolowo University, Ile-Ife, Nigeria. The two onion bulbs were rinsed in 5% HOCL for few minutes and cut into seven sections each, with a sterile knife under aseptic conditions to produce fourteen sections in total. The first seven sections from the first onion bulb were placed on a sterile stainless-steel plate and stored at room

temperature for seven days, while the remaining seven sections of the second bulb were placed on a sterile stainless-steel plate and stored for seven days in the refrigerator [15].

2.2 Isolation of the Microorganisms Associated with Onion Bulbs in Storage

Microbial samples from the onion surface were collected daily for 7 days by using sterile swab sticks that have been dipped into peptone water. The swabs were thereafter streaked onto the surface of Petri dishes containing nutrient agar (NA), Mannitol Salt Agar (MSA), MacConkey agar (MAC) for bacterial isolation and Potato Dextrose Agar (PDA) for fungi cultivation. The NA, MSA, and MAC plates were incubated at 37°C for 24 hours, while the PDA plates were incubated at 25°C for 5 days [15].

2.3 Characterization and Identification of the Bacterial and Fungal Isolates

Each of the bacterial isolates from the different plates was streaked onto the surface of freshly prepared NA plate and incubated at 37°C for 24 hours. The bacterial isolates associated with the stored onion bulbs were characterized using their reactions to Gram's staining, their different morphological and biochemical characteristics [22,23] and identified as described [24]. The fungal isolates obtained were each sub-cultured onto freshly prepared PDA plates and incubated at 25°C for 5 days. A drop of lactophenol cotton blue stain was deposited on a clean slide, with the aid of a mounting needle, a thin film of aerial mycelia from the representative fungal cultures was removed and placed on a drop of the lactophenol cotton blue stain. The hyphae of the mycelium were gently teased out on the slide with the needle, and a coverslip was placed carefully on the slide with little pressure, to avoid air bubbles. The slide was then mounted and viewed under the light microscope with ×10 and ×40 objective lenses [25]. The sketch of each isolate was carefully taken under the microscope and identified by comparing with that of Barnett and Hunter's manual of *Illustrated Genera of Imperfecti Fungi* [26]. The identification was based on cultural and morphological features, as the colour and average diameter of the mycelia were measured and recorded.

2.4 Pathogenicity Test

The outer scales of the healthy onion were removed and the inner tissues were swabbed

with cotton wool pre-soaked in 1% sodium hypochlorite (NaOCl) to remove any contaminant on the surface. The onion samples were dried by cleaning with sterile cotton wool. A sterile cork-borer of a diameter of 2 mm was used to lacerate each of the onion bulbs. The lacerated bulbs were each inoculated with 0.25 ml of standardized inoculum (0.5 McFarland Standard) of the identified bacterial isolates. Thereafter, the lacerated portion was sealed with petroleum jelly and the bulbs incubated at 37°C for 7 days [27]. Another onion bulb that has undergone similar treatment (only that it was not inoculated with any of the bacterial isolates) was incubated at 37°C for 7 days) served as the control. The inoculated onion bulbs were each observed daily for 7 days and the measurement of the diameter of the disrupted parts from the point of inoculation on the deteriorating onion bulbs were recorded on the last day [27].

3. RESULTS

3.1 Identification of the Bacteria Isolated from the Onion Bulbs

Thirty-five (35) bacterial isolates were cultured and four (4) different bacterial genera were identified from the fourteen onion bulb sections sampled. All the thirty-five bacterial isolates identified were Gram-positive; 11 (31.4%) *Staphylococcus* spp., 9 (25.7%) *Micrococcus* spp., 8 (22.9%) *Bacillus* spp. and 7 (20%) *Flavobacterium* spp., as shown in Table 1.

Table 1. The Frequency of the bacterial isolates

Bacterial isolates	Number	Percentage dominance
<i>Staphylococcus</i> spp.	11	31.4%
<i>Bacillus</i> spp.	9	25.7%
<i>Flavobacterium</i> spp.	8	22.9%
<i>Micrococcus</i> spp.	7	20%
Total	35	100%

3.2 Characteristics of the Fungi Isolated from the Onion Bulbs

The fungal isolates identified are as shown in Table 2. A total of seven fungi of three different genera were identified, which included 5 (71.4%) *Aspergillus fumigatus*, 1 (14.3%) *Gibellula suffulta* and 1 (14.3%) *Hirsutella saussueri*.

3.3 Establishment of the Pathogenicity of the Bacterial Isolates

The pathogenicity profiles of the bacterial isolates are as shown in Table 3. The highest was *Flavobacterium* spp. (28 mm), followed by *Staphylococcus* spp. (17 mm), *Bacillus* spp. (15 mm), while *Micrococcus* spp. (14 mm) was the least.

4. DISCUSSION

This study isolated and identified the microorganisms associated with soft rot in onion bulbs, and determined the pathogenicity of the bacterial isolates obtained.

The bacterial isolates identified included; *Micrococcus* spp., *Staphylococcus* spp., *Bacillus* spp. and *Flavobacterium* spp. *Bacillus cereus*, *Salmonella*, *Escherichia coli* and *Flavobacterium* spp. are naturally present in some soil, hence the isolation of *Bacillus* spp. and *Flavobacterium* spp. in this study is not a rare occurrence [28,17]. However, the bacterial isolates obtained in this

study were slightly different from the ones identified in deteriorating onion bulbs stored at Annapolis valley in Canada by the previous findings of Yurgel et al. [15]; aside bacteria like *Flavobacterium* spp., they also identified *Pseudomonas* spp., *Enterobacteria* spp., *Erwinia* spp., and *Acinetobacter* spp., among others. This difference in the bacterial community observed in the study in Canada and our study in Nigeria could be due to the difference in the genotype of the cultivated onion bulbs, climatic factors and cultivating environment [15].

Orpin et al. [29] previously isolated *Staphylococcus* spp. and *Bacillus* spp. from onion bulbs sold in Dutsinma Metropolis, Nigeria. The isolation of *Staphylococcus* spp. and *Micrococcus* spp. in our study is not strange since they are known inhabitants of the human skin, hence, their presence on the stored onion bulbs could be as a result of handling by farmers and traders. It has been reported that improper handling and hygiene practices might lead to the contamination of food, thereby, having adverse effects on the health of the consumers [30].

Table 2. Diagrammatic sketch, brief description, average diameter and identity of the fungal isolates

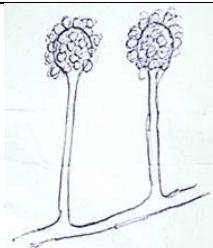
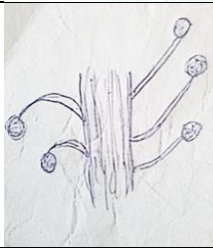

Code	Diagrammatic sketch	Brief description	Average diameter (mm)	Identity
NTE ₁		Black, long, upright, spiral, radiating at the apical, globose, ovoid, simple, non-septate, clavate swollen.	64	A. <i>Fumigatus</i>
NTE ₂		Dark green, conidiophore Short, slender, globose, dense. Conidia fusoid to ellipsoid, cylindrical bearing prophialides broadly enlarged, single and simple.	37	<i>Gibellula suffulta</i>
NTE ₃		Light green spores on white mycelium, slender conidiophore composed of loose longitudinal hyphae, terminal cell, apex enlarged, ovoid L-celled and oblong to cylindrical.	43	<i>Hirsutella sausseri</i>

Table 3. Pathogenicity test of the bacterial isolates

Bacterial isolate	The diameter of rot from the point inoculation (mm)	Physical characteristics of rot
<i>Flavobacterium</i> spp.	28	Visible discolouration and softening of the tissue. Extension of rot to head and neck of the bulb with a heavy load of black spores.
<i>Staphylococcus</i> spp.	17	Brown discolouration and deteriorating inner layer of tissue sites closest to the bore site.
<i>Bacillus</i> spp.	15	Slime present alongside discolouration and weakening of tissues. Heavy load of visible white colonies with black spores.
<i>Micrococcus</i> spp.	14	Yellowing of ring region with characteristic drying up of the topmost tissue layer.

Bulb rot in onion has been attributed to both fungi and bacteria. In this study, the fungi isolated included; *Aspergillus fumigatus*, *Gibellula suffulta* and *Hirsutella saussueri* which were different from the fungal isolates reported in a study conducted by Akintobi et al. [31], where *Aspergillus flavus*, *A. niger*, *Fusarium solani*, *Penicillium digitatum* and *Rhizopus stolonifera* were the common fungal isolates identified as causal agents of onion bulb spoilage. However, a study conducted by Shehu and Muhammad [32] agrees with our study as *Aspergillus fumigatus* was also reported to be among the fungi associated with the postharvest deterioration of onion bulbs that were sold at different markets in Sokoto, Nigeria. The deterioration of food items caused mainly by the activities of the microorganisms in food items renders them undesirable to consumers. Thus, spoilage of commercially purchased onion bulb is a cause for concern especially by fungi acting on the bulbs in storage, particularly in the tropics [27]. Apart from toxin production, the presence of fungi on the onion bulbs eventually leads to disease development in the field when the infected bulbs are planted in the following cropping season [32].

The prominent bacteria isolates implicated in onion bulb rot include; *Erwinia* spp., *Pseudomonas aeruginosa*, *Enterobacter* spp., *Bacillus* spp., *Escherichia coli*, *Staphylococcus* spp., among others [33,34,15]. The pathogenicity test carried out on the bacterial isolates obtained in this study reveals that the bacterial isolates were able to successfully cause rot in healthy onion bulb under favourable conditions, just like their fungal counterparts that have been

established previously to cause bulb rot in onion [32,27,8]. It has been shown that the bacterial bulb rot is caused by *Bacillus amyloliquefaciens* in a study conducted in Korea with dark brown lesions observed in the rotten onion [34], likewise, the *Bacillus* spp. isolated in this study produced rot in onion having characteristic symptoms of slime with discolouration, weak tissues and a heavy load of visible white colonies with black spores. The development of bacterial bulb rot in onion can be controlled by altering the storage conditions to make it unfavourable for the bacteria to thrive [34].

Some of the bacteria isolated in this study have been shown to cause rot in onion bulb apart from the report that they can cause foodborne diseases outbreak. Callejón et al. [35] reported that *Staphylococcus* spp. and *Bacillus* spp. were responsible for foodborne outbreaks in the United States and Europe. The contamination of these fresh produce by bacterial pathogens poses a potential health risk, hence, they should be thoroughly washed or processed to minimize the risk of foodborne diseases.

5. CONCLUSION

It has become very important for every individual or group of individuals involved in the chain of distribution to take necessary and appropriate precautions to prevent the microbial contamination of onions; deteriorating onion bulbs should be separated from the fresh ones to avoid cross-contamination. The diversity of microbial community implicated in the bulb rot of onion as presented in this study can help in the postharvest management of the disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
 The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/61173>