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Isolation and Phenotypic Characterization of Culturable Bacteria Associated with Spoiled Onion Bulbs (*Allium cepa* L.) Sold in Selected Markets of Dutse Metropolis, Jigawa State, Nigeria

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ABSTRACT
Spoilage of onion bulbs causes economic losses and may pose health risks, and bacterial contamination is one of its main contributors. This study isolated and characterised culturable bacteria associated with spoiled onion bulbs sold in Dutse metropolis, Jigawa State, Nigeria. Ten spoiled onion samples (five each from Hakimi Street and Sabuwar Kasuwa markets) were aseptically prepared, serially diluted, and plated on nutrient agar. Isolates were characterised by colony morphology, Gram staining, microscopy, and biochemical tests. Colony counts ranged from 5.5×10^4 CFU/mL (H5) to 4×10^5 CFU/mL (H4) among Hakimi Street samples and from 5.5×10^4 CFU/mL (SW3) to 4.5×10^5 CFU/mL (SW4) among Sabuwar Kasuwa samples. Phenotypic characteristics indicated *Staphylococcus* spp. (30%), *Micrococcus* spp. (30%), *Escherichia coli* (20%), and *Klebsiella* spp. (20%). All isolates were catalase positive; the *Staphylococcus* isolates were coagulase positive, whereas *E. coli*, *Micrococcus* spp., and *Klebsiella* spp. were coagulase negative. Because identification relied on phenotypic characteristics only, the isolates are presumptive, and their occurrence indicates association with spoiled onions rather than proof that they caused the spoilage. Further studies using molecular identification and pathogenicity tests are recommended to establish species-level identities and the organisms responsible for spoilage.

Keywords: Onion, *Allium cepa,* Spoilage, Bacteria, Dutse, Jigawa State

INTRODUCTION

Onion (*Allium cepa*) is a significant vegetable crop planted all over the world for both human consumption and farmer profit (FAO, 2012; Habtamu, 2017). It is a member of the Alliaceae family and is farmed for its seasoning- and flavouring-filled bulbs, which are used every day in every home. Onion is a valuable food since it contains carbohydrates, vitamins, and minerals (National Onion Association, 2017). Nigeria has grown significantly in onion production in recent years, most likely as a result of greater awareness of its nutritional value. When bulbs are inadequately stored, they degrade and decay during preservation, rendering them unusable, as a consequence of insufficient dryness of the bulbs prior to storage or the presence of storage microorganisms on the bulbs prior to storage (Schroeder et al., 2012). Onion has been found to harbour pathogens such as *Pseudomonas* sp., *Staphylococcus* sp., and *Erwinia* sp., which frequently affect germination and crop output (Orpin et al., 2017).

Microbial deterioration has emerged as a significant factor in the failure of sensory quality and shelf life of preserved onions (Roopa et al., 2014). Onion storage results in significant quality and quantity losses, and storing onion bulbs has become a major issue in tropical nations (Kughur et al., 2015). Onion growers estimate losses of up to 40% when weather conditions during the growing season favour bacterial growth (Pfeufer et al., 2015). When food is improperly harvested and handled afterward, it degrades in quality, losing its look, flavour, and nutritional content before it reaches consumers. To minimize the postharvest loss of onion, it is important to design management action and conduct research on the microorganisms responsible for spoilage.

During the metabolic process of onion rotting, changes in the vegetable's sensory properties render it unsuitable or unpleasant for human eating; the texture, flavour, aroma, or look of spoiled onions may change, making them unappealing to consumers (Rewas, 2015). Onion spoilage and postharvest loss can happen from improper onion storage after harvest, which allows for the growth of rotting bacteria and fungi (Samuel and Ifeanyi, 2015). Spoilage bacteria are frequently introduced into the crop while growing in the field, during harvest and postharvest processing, during storage and distribution, or on the onion seed itself (Olani and Fikre, 2010).

Dry matter content, pungency, skin colour, skin quality, the length of the variety's natural dormancy period, the fertilizer and water regime during cultivation, treatment of sprout suppressants and fungicides, and postharvest factors (time and method of harvesting, curing, storage conditions, and packing materials) all affect the shelf life of onions (Erin, 2010; Getenesh et al., 2015). The majority of sources of microbial contamination and subsequent vegetable rotting appear to be bacteria that live on the surfaces of raw, intact food; bacteria on an onion's surface may therefore be to blame for spoiling the onion and the resulting postharvest loss (Dada and Olusola-Makinde, 2015). Raw onions can degrade due to physical causes, the onion's own enzymes, microbial activity, or a combination of these. Because the bacteria are so small, even tiny wounds, such as those created by sucking or chewing insects, can allow entry; once inside the onion, the bacteria multiply between the plant cells.

A number of bacterial onion spoilages pose a challenge to onion farmers worldwide, resulting in losses to bulbs during harvest, storage, and the marketing chain. *Burkholderia cepacia*, *Pantoea ananatis*, and *Enterobacter cloacae* are

among the most significant bacteria that harm onions (Carr et al., 2010; Brady et al., 2013). Onions are frequently affected near or at harvest, and the bacteria typically penetrate the plant through wounds caused by wind-driven hail, ploughing, mechanical damage during harvesting, or excessive moisture during storage (Pfeufer et al., 2015).

Bacterial soft rot can destroy fleshy crops including onions, and is most commonly identified by a soft, wet, cream-to-tan colour encircled by a dark brown to black ring; onions in storage or transit are most frequently affected. *Erwinia*, *Pectobacterium*, and *Pseudomonas* are the gram-negative bacterial species that most frequently cause bacterial soft rot (Mohamed et al., 2011). *Enterobacter* bulb rot is another reason for onion loss during storage: when the onion bulb is split in half, the decayed tissue takes on a dark colour and gives the bulb the appearance of a dirty ring; *Enterobacter cloacae* is the organism responsible (Humann et al., 2011; Liu et al., 2016). Slippery skin, caused by *Burkholderia gladioli* pv. *alliicola* (and related to *Burkholderia cepacia*), is another reason onions lose product during storage and harvest; the bulbs may seem healthy in the early stages except for a softening of the neck tissue (Schroeder et al., 2012).

The loss of onions due to bacterial spoilage can be attributed to improper postharvest handling procedures such as inadequate temperature control, lack of sanitary practices, and prolonged storage period (Kughur et al., 2015). This can lead to the growth of bacteria on the surface of the onions, causing them to deteriorate and become unmarketable. Increased postharvest losses not only limit onion availability but also raise the cost of produce per unit, limiting access for the majority of the community. Consumption of raw spoiled onion may cause severe stomach ache due to the presence of members of *Enterobacter*iaceae.

The study of onion spoilage is important for several reasons. First, it can help improve the economic viability of onion production by reducing product loss due to spoilage. Second, it can help improve the safety of onions for human consumption by identifying and controlling the growth of potentially harmful bacteria. Third, it can help increase the nutritional value of onions by identifying and retaining beneficial compounds during storage and transport. Fourth, it can help improve the sustainability of onion production by reducing the resources required to produce and maintain fresh onions.

The aim of this study was to identify and isolate the bacteria associated with spoiled onion (*Allium cepa*). The specific objectives were to isolate and identify bacteria associated with spoiled onion, to determine the percentage occurrence of each isolated bacterium, and to analyze bacterial occurrence using a simple statistical approach.

MATERIALS AND METHODS

Sample Collection

The onion bulbs were collected from two distinct market places, namely Hakimi Street and Sabuwar Kasuwa markets. The samples were packed in sterile bags and sent to the Microbiology Laboratory of the Department of Microbiology, Federal University Dutse, Jigawa State. Samples from Hakimi Street market were designated H1–H5, and samples from Sabuwar Kasuwa market were designated SW1–SW5 (five samples from each market, ten in total).

Media Preparation

Nutrient agar (NA) was used for isolation of bacteria occurring on spoilt onions. The medium was prepared according to the manufacturer's instructions and sterilized at 121°C for 15 minutes, then allowed to cool to 37°C (Cheesbrough, 2006).

Sample Preparation and Serial Dilution

One gram of spoiled onion tissue was cut with a sterile razor blade from each sample and transferred separately into a sterile mortar and pestle. Sterile distilled water was added and the tissue was crushed. One millilitre of the resulting homogenate was added to 9 mL of sterile Nutrient broth in a universal bottle, and serial dilution was carried out from each sample using this ten-fold dilution procedure (Cheesbrough, 2006; Darlington et al., 2024).

Inoculation and Isolation of Bacteria

About 0.1 mL of the aliquot from the serial dilution of each sample was added to the media (Nutrient agar), and each plate was poured using sterile technique and allowed to solidify. This procedure was repeated for each sample. The inoculated plates were incubated, inverted, at 37°C for 24 hours (Cheesbrough, 2006). This was done for dilutions of 10^{-3} and 10^{-4} for all samples.

Determination of Colony Forming Units

After incubation, the number of colonies that formed on each agar plate was counted, and the colony-forming units (CFU) per millilitre of the original sample were calculated from the counted plate and the applied dilution factor.

Gram Staining

Gram staining was used to group the isolates into one of two major groups, Gram-positive or Gram-negative, based on the composition of their cell wall and cell membrane (Bartholomew and Mittwer, 1952; Thairu et al., 2014). A smear was made on a clean glass slide by taking a loop of growth from an isolated colony and mixing it with a drop of distilled water. The smear was heat-fixed using a Bunsen burner. The slide was flooded with the primary stain, crystal violet, for 1 minute, then rinsed with distilled water. Iodine was applied for another 1 minute, followed by a rinse. Ethanol was then applied as a decolorizer for 10 seconds, followed by a rinse. Finally, the counterstain safranin was applied for 1 minute, followed by a rinse, and the slide was air-dried before viewing under the microscope (Cheesbrough, 2006).

Microscopy

Stained smears were viewed under the microscope at x10 to x40 objective lenses to detect Gram-positive or Gram-negative bacteria, observed as cocci or bacilli. Gram-positive bacteria were identified by a dark purple colour (retained crystal violet), while Gram-negative bacteria appeared pale to dark red (from the safranin counterstain). Gram reactions are reported as positive (+) or negative (−) in Tables 3–5.

Biochemical Tests

Biochemical tests were carried out on the isolates as follows (Adebami et al., 2026; Cheesbrough, 2006; Phillips et al., 2012). The tests were catalase (CAT), coagulase (COA), indole (INDO), citrate (CIT), methyl red (MR), and Voges–Proskauer (VP); results are reported as positive (+) or negative (−) in Tables 4 and 5.

Catalase test: a small amount of bacterial growth was transferred to a drop of hydrogen peroxide on a clean glass slide using a sterile wire loop. Bubbling indicated a positive result; absence of bubbling indicated a negative result (Cheesbrough, 2006).

Coagulase test: a drop of plasma and a suspension of bacterial cells were prepared on a microscope slide. Clumping of the

bacterial cells in the presence of plasma indicated a positive result (Cheesbrough, 2006).

Indole test: used to identify bacteria capable of producing indole from tryptophan via tryptophanase. Kovacs reagent was added to the medium after incubation; a red colour change indicated a positive result (Cheesbrough, 2006).

Methyl red (MR) test: the pH indicator methyl red was added to a 48-hour culture. A red colour indicated a positive result; yellow indicated a negative result (Cheesbrough, 2006).

Voges–Proskauer (VP) test: performed on the same MR/VP broth. Barritt's reagent A (alpha-naphthol) and Barritt's reagent B (potassium hydroxide) were added after 48 hours of incubation. A red colour indicated a positive result; no colour change or a copper colour indicated a negative result (Cheesbrough, 2006).

Citrate utilization test: used to determine the ability of an organism to use citrate as its sole carbon source. A positive result was indicated by growth with blue colouration; a negative result showed no growth and the medium remained green (Cheesbrough, 2006).

Statistical Approach

Simple percentage was used to determine the occurrence frequency of the bacteria isolated from the spoilt onion samples.

RESULTS AND DISCUSSION

Total Colony Count (Aerobic Bacterial Count)

Table 1 shows the total colony count (CFU/mL) for the ten onion samples collected from Hakimi Street and Sabuwar Kasuwa markets (five from each market), plated at dilutions of 10^{-3} and 10^{-4}.

The highest bacterial colony count among the Hakimi Street samples was recorded in H4 (4×10^5 CFU/mL) and the lowest in H5 (5.5×10^4 CFU/mL). The highest count among the Sabuwar Kasuwa samples was recorded in SW4 (4.5×10^5 CFU/mL) and the lowest in SW3 (5.5×10^4 CFU/mL).

Table 1: Total Colony Count from Samples Collected from Hakimi Street and Sabuwar Kasuwa Market

S/N	Sample ID	Dilution Plated	Colony Count (CFU/mL)
1	H1	10^{-3}	8×10^4
2	H2	10^{-4}	3.5×10^5
3	H3	10^{-3}	6×10^4
4	H4	10^{-4}	4×10^5
5	H5	10^{-3}	5.5×10^4
6	SW1	10^{-3}	7×10^4
7	SW2	10^{-4}	4.2×10^5
8	SW3	10^{-3}	5.5×10^4
9	SW4	10^{-4}	4.5×10^5
10	SW5	10^{-3}	6.3×10^4

Identification of Bacterial Isolates from Spoiled Onion

Colony morphology, Gram staining, microscopic identification, and biochemical characterization were used to identify the bacteria isolated from the spoilt onion samples. Following these tests, the organisms suspected to be present were Staphylococcus spp., Escherichia coli, Micrococcus spp., and Klebsiella spp. Identification was based on phenotypic (cultural, microscopic and biochemical) characteristics only and should therefore be regarded as presumptive.

Table 2: Cultural Characteristics of Isolated Colonies from Hakimi Street and Sabuwar Kasuwa Market Isolates on Nutrient Agar

S/N	Sample ID	Form	Margin	Elevation
1	H1	Irregular	Entire	Raised
2	H2	Punctiform	Erose	Flat
3	H3	Irregular	Lobate	Raised
4	H4	Rhizoid	Entire	Flat
5	H5	Irregular	Filamentous	Flat
6	SW1	Rhizoid	Lobate	Raised
7	SW2	Punctiform	Erose	Flat
8	SW3	Irregular	Lobate	Raised
9	SW4	Irregular	Entire	Flat
10	SW5	Irregular	Undulate	Flat

Table 3: Microscopic Observation of Bacterial Isolates from Hakimi Street and Sabuwar Kasuwa Market

S/N	Sample ID	Gram RXN	Colour	Shape/Arrangement	Suspected Organism
1	H1	+	Purple	Cocci, in clusters	*Staphylococcus* spp.
2	H2	−	Pink	Bacilli, rod-shaped	*Escherichia coli*
3	H3	+	Purple	Cocci, in clusters	*Micrococcus* spp.
4	H4	−	Pink	Bacilli, rod-shaped	*Klebsiella* spp.
5	H5	+	Purple	Cocci, in clusters	*Staphylococcus* spp.
6	SW1	−	Pink	Bacilli, rod-shaped	*Escherichia coli*
7	SW2	+	Purple	Cocci, in clusters	*Micrococcus* spp.
8	SW3	−	Pink	Bacilli, rod-shaped	*Klebsiella* spp.

S/N	Sample ID	Gram RXN	Colour	Shape/Arrangement	Suspected Organism
9	SW4	+	Purple	Cocci, in clusters	*Staphylococcus* spp.
10	SW5	+	Purple	Cocci, in clusters	*Micrococcus* spp.

Table 4: Biochemical Test of Bacterial Isolates from Hakimi Street Spoiled Onion

Sample ID	Gram RXN	CAT	COA	INDO	CIT	MR	VP	Organism
H1	+	+	+	−	+	+	+	*Staphylococcus* spp.
H2	−	+	−	+	−	+	−	*Escherichia coli*
H3	+	+	−	+	−	+	−	*Micrococcus* spp.
H4	−	+	−	−	+	−	+	*Klebsiella* spp.
H5	+	+	+	−	+	+	+	*Staphylococcus* spp.

Table 5: Biochemical Test of Bacterial Isolates from Sabuwar Kasuwa Market Spoiled Onion

Sample ID	Gram RXN	CAT	COA	INDO	CIT	MR	VP	Organism
SW1	−	+	−	+	−	+	−	*Escherichia coli*
SW2	+	+	−	+	−	+	−	*Micrococcus* spp.
SW3	−	+	−	−	+	−	+	*Klebsiella* spp.
SW4	+	+	+	−	+	+	+	*Staphylococcus* spp.
SW5	+	+	−	+	−	+	−	*Micrococcus* spp.

Table 6: Determination of Percentage Occurrence

Organism	Occurrence	Percentage
Staphylococcus spp.	3	30%
Micrococcus spp.	3	30%
Escherichia coli	2	20%
Klebsiella spp.	2	20%
Total	10	100%

Discussion

The presence of microorganisms in the onion bulbs is attributed to environmental conditions, the state of handling and processing, and the storage facilities used for the onion bulbs (Aminu and Ali, 2017; Dada and Olusola-Makinde, 2015). These microorganisms have been known to cause disease in humans (Krishna and Sarita, 2016). The predominance of Gram-positive cocci (*Staphylococcus* spp. and *Micrococcus* spp.), together with the presence of Gram-negative enteric bacilli (*Escherichia coli* and *Klebsiella* spp.), suggests that contamination of the bulbs may have occurred from more than one source, such as human handling and environmental reservoirs (Adebami et al., 2026).

Other studies have reported that *Staphylococcus* spp. is a major cause of onion spoilage (Ibemologi and Tolulope, 2022; Orpin et al., 2017), consistent with the findings of this study, in which *Staphylococcus* spp. was among the most frequently occurring suspected organisms. *Staphylococcus* spp. is commonly found on the human body in areas such as the hand, hair, and mouth, and has also been isolated from foods (Darlington et al., 2024; Resch et al., 2008). *Escherichia coli* is a Gram-negative bacterium that can play a role in onion spoilage; onions can become contaminated with *E. coli* through contaminated water, soil, or improper handling during cultivation, harvesting, or processing (Doyle and Erickson, 2008).

Micrococcus spp. is another organism reported to be responsible for onion spoilage, possibly entering through cuts on the onion during harvest, as it is readily found in the soil. This corresponds with the findings of this study, in which *Micrococcus* spp. was, together with *Staphylococcus* spp., the most frequently occurring organism. *Escherichia coli* is also suspected to cause spoilage in onions from this study; this can be attributed to the fact that such organisms are widely distributed in a variety of environmental habitats, including soil and ambient water (rivers, streams), so their occurrence in the onion may stem from these sources. *Staphylococcus* spp. and *Escherichia coli* have likewise been isolated from vended ready-to-eat foods in Rivers State, Nigeria (Darlington et al., 2024), and *Staphylococcus*, *Micrococcus*, Escherichia, and *Klebsiella* species from the air of primary health care centres in Ogun State, Nigeria (Adebami et al., 2026), which is consistent with the transfer of these bacteria through human contact and the environment.

Identification in this study was based on cultural, microscopic and biochemical characteristics only, so the isolates are presumptive. Some results, such as the indole- and methyl red-positive reactions recorded for Micrococcus spp., are atypical for the genus and highlight the need for confirmation by additional biochemical tests or molecular methods. In addition, because the isolates were obtained from bulbs that were already spoiled, their occurrence shows association with spoiled onion but does not establish that they caused the

spoilage; this would require pathogenicity (re-inoculation) tests.

Conclusion

The results of this study revealed that the onions purchased from Hakimi Street and Sabuwar Kasuwa markets had some degree of bacterial contamination, showing that onions harbour different bacteria associated with spoilage. *Staphylococcus* spp. and *Micrococcus* spp. were the most frequently occurring bacteria (30% each) in the spoilt onions. The result of this study shows that spoilt onion bulbs are a reservoir of bacteria associated with spoilage, which could result from exposing the onions to a poor environment such as poor packaging, transportation, and storage. Appropriate control measures must be employed during the harvesting, processing, transporting, and handling of onions. The presence of spoilage bacteria in onions may be an important contributor to postharvest loss. To minimize postharvest loss of onion, preservation methods at the harvesting site, in the transport chain, and at the marketing area should be considered to minimize contamination. It is important to improve knowledge about these spoilage bacteria and their activities in order to find rapid methods of preservation and technological solutions (storage and packaging conditions) to slow their growth and metabolic activities. Consumption of raw onion without prior heat treatment should be discouraged. It is therefore recommended that onion bulbs be handled gently during harvesting to avoid bruises, that contaminated water be avoided for irrigation, that good storage facilities be provided for onion bulbs, and that raw onions be washed properly before consumption.

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