

Isolation and Biochemical Analysis of Microorganisms Found in Stored Maize

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Abstract

Stored maize is susceptible to microbial contamination, which poses significant risks to food safety and public health. This study investigated the isolation, enumeration, and biochemical characterization of microorganisms found in stored maize samples. Serial dilutions of the samples were inoculated onto Nutrient agar, MacConkey agar, and Sabouraud dextrose agar using spread techniques. Bacterial counts ranged from 1.12×10^7 to 6.67×10^7 CFU/ml, while fungal counts ranged from 6.27×10^7 to 6.67×10^7 CFU/ml. Biochemical and morphological analyses identified *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* among the bacterial isolates, while fungal isolates included *Aspergillus fumigatus*, *Aspergillus niger*, and *Rhizopus microsporus/stolonifer*. The presence of both pathogenic and spoilage organisms highlights the potential health hazards of improperly stored maize and underscores the importance of effective storage practices and microbial monitoring to ensure food safety.

Keywords: Stored maize; *Staphylococcus aureus*; *Escherichia coli*; *Pseudomonas aeruginosa*; *Aspergillus fumigatus*; *Aspergillus niger*; *Rhizopus microsporus*; e.

Received: 1/13/2026

Accepted: 3/13/2026

Published: 3/23/2026

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1.Introduction

Maize (*Zea mays*) is one of the most important staple crops globally, serving as a primary source of food, feed, and industrial raw material. It is cultivated widely across Africa, Asia, and the Americas, with Nigeria ranking among the largest producers in sub-Saharan Africa[1-5] .Despite its economic and nutritional significance, maize is highly vulnerable to microbial contamination during storage[5-8].Poor storage practices, high humidity, elevated temperatures, and inadequate aeration influence the conditions for the growth of bacteria and fungi that affect the maize quality and safety[7, 9].

Microbial contamination of stored maize has been reported worldwide, with fungi such as *Aspergillus*, *Penicillium*, and *Fusarium* producing mycotoxins like aflatoxins and fumonisins [5, 7, 9, 10].Bacterial contaminants such as *Bacillus*, *Pseudomonas*, and *Enterobacter* species also contribute to spoilage, which reduces the nutritional value of maize and sometimes produces harmful metabolites[5, 7, 9, 11] .These microorganisms not only affect food safety but also lead to significant economic losses due to reduced marketability and rejection of contaminated grains in international trade. Also, these microorganisms pose serious health risks, including liver cancer, immune suppression, and stunted growth in children.

In Nigeria, where maize is a dietary staple and a major source of livelihood, microbial contamination of stored maize remains a pressing challenge[5, 12] .Farmers and traders often rely on traditional storage methods that lack adequate protection against microbial invasion. As a result, consumers are exposed to contaminated maize products, raising public health concerns[5, 12] .Its storage is often challenged by microbial contamination, which leads to spoilage, reduced nutritional quality, and potential health risks.

Despite the importance of maize in human diets and the economy, limited research has comprehensively identified and characterized the microorganisms associated with stored maize in many sub-Saharan regions. Furthermore, the biochemical activities of these microorganisms, such as enzyme production and toxin synthesis, are poorly understood, yet they are the cause of maize spoilage and food safety risks. Without proper identification and biochemical analysis of these microorganisms, effective interventions for safe storage and control of contamination cannot be developed. Therefore, there is a pressing need to isolate and biochemically characterize the microorganisms present in stored maize to understand their spoilage mechanisms and potential health implications. This knowledge will contribute to preceding findings and provide a foundation for improved storage practices, food safety regulations, and strategies to reduce post-harvest losses.

This research, based on biochemical analysis of microorganisms isolated from stored maize, will provide critical insights into their metabolic activities, pathogenic potential, and spoilage mechanisms. Enzyme production (such as amylase, protease, and lipase) by these microbes accelerates the breakdown of starch, proteins, and lipids in maize, leading to deterioration[5, 13].Understanding these biochemical properties is essential for developing effective control strategies, including improved storage technologies, biocontrol agents, and food safety regulations.

Research on the isolation and biochemical characterization of microorganisms in stored maize is therefore vital

for identifying the specific spoilage organisms present, assessing their potential risks, and proposing sustainable interventions to safeguard food quality and security. The objectives are

- i. To isolate microorganisms using selective culture media.
- ii. To identify bacterial and fungal isolates through morphological and biochemical tests.
- iii. To assess the potential implications of these microorganisms on maize quality and food safety.

2.Methods

2.1 Sample Collection Point

Stored maize samples were collected from foodstuff markets at Iddo Sarki, Abuja, Nigeria. The study was experimental-based, in which the isolation and identification of bacterial and fungal organisms associated with stored maize were conducted. The practical was conducted in the Microbiology Laboratory, Department of Microbiology, University of Abuja. The stored maize samples were ground into powder and soaked in water for seven days. Two samples were prepared for bacterial and fungal isolation

2.2 Serial Dilution

The samples were serially diluted. nine-fold serial dilutions labeled from 10^0 - 10^{-8} were prepared using sterile distilled water. The serial dilution was achieved by mixing the sample thoroughly and transferring 1ml into 9ml of the diluent to make 10^{-1} [14]. This pattern was repeated to obtain dilutions of 10^{-5} and 10^{-6} , which were used for the research. These dilutions were used for both bacterial (named Sample B1 and B2) and fungal (F1 and F2) inoculations to standardize microbial load and facilitate isolation of distinct colonies.

2.3 Media Preparation and Isolation

Culture media were prepared according to the manufacturer's instructions. Two separate samples were prepared for bacterial and fungal isolation to ensure the reproducibility and accuracy of the results.

For bacterial isolation, 0.1 mL of Sample B1 (10^{-5}) and B2 (10^{-6}) were inoculated into Nutrient Agar and MacConkey Agar.[14, 15] Each sample was streaked onto the agar plates using the streak plate method to obtain discrete colonies. The plate was allowed to gel properly, sterilized at 121°C for 15 minutes, and cooled to $45\text{--}50^{\circ}\text{C}$ before being dispensed aseptically into Petri dishes. The plates were incubated at 37°C for 24–48 hours, after which bacterial growth was observed and recorded. For fungal isolation, 0.1 mL of Sample F1 (10^{-5}) and F2 (10^{-6}) were inoculated with Sabouraud Dextrose Agar (SDA) plates using the streak plate method to facilitate colony separation. [16]The plates were incubated at 25°C for 3–5 days[17, 18].

2.4 Plate Count

After incubation, colonies on Nutrient and MacConkey agar plates were examined. Plates with 30–300 colonies were counted, while those with colonies too numerous to count (TNTC) were discarded. Fungal colonies on

Sabouraud dextrose agar (SDA) plates were also counted. The viable count was calculated using the formula:

$$\text{CFU/mL} = \frac{\text{mean colony count}}{0.1} \times 10^{\text{dilution factor}}$$

2.5 Isolation of Pure Culture

After incubation, colonies with distinct cultural characteristics were selected using a sterile wire loop. These were streaked onto fresh Nutrient, MacConkey, and SDA agar plates to obtain pure cultures. Each pure isolate was subsequently sub-cultured onto agar slants in bijou bottles and preserved as stock cultures. Three distinct colonies (isolates) were obtained from bacterial culture and fungal culture separately. For the bacterial isolates, they were named B10, B11, and B12, while the fungal isolates were named F10, F11, and F12.

2.5 Identification of Bacterial Isolates

Bacterial identification followed the classification scheme in Bergey's Manual of Determinative Bacteriology, based on morphology, physiology, growth requirements, and biochemical characteristics. Colony morphology, Gram staining, and biochemical tests were employed for identification [14].

2.5.1 Gram Staining

Smears were prepared on glass slides, heat-fixed, and sequentially stained with crystal violet (1 min), Gram's iodine (1 min), decolorized with 95% ethanol (5–10 sec), and counterstained with safranin (45 sec).[14] Slides were rinsed, blot-dried, and examined under oil immersion using a light microscope.

2.5.2 Biochemical Tests for Bacterial Isolates

The following assays were performed to characterize bacterial isolates from stored maize.

1. **Catalase Test:** A drop of 3% hydrogen peroxide was added to a fresh bacterial colony on a clean slide. Immediate effervescence (bubble formation) indicated the presence of catalase enzyme, which breaks down hydrogen peroxide into water and oxygen.
2. **Coagulase Test:** A portion of the isolate was emulsified in normal saline to form two thick suspensions on a clean glass slide. Human or rabbit plasma was added to one suspension, while the second suspension served as a control without plasma. The mixtures were gently agitated and observed for clumping within 10 seconds. The presence of visible clumping in the plasma-treated suspension, in contrast to the control, was interpreted as a positive coagulase reaction.
3. **Oxidase Test:** Bacterial colonies were smeared on filter paper impregnated with oxidase reagent (tetramethyl-p-phenylenediamine). A deep purple coloration within 30 seconds indicated a positive oxidase reaction, confirming the presence of cytochrome oxidase.
4. **Motility Test:** Isolates were inoculated into semi-solid agar medium and incubated at 37 °C for 24 hours. Diffuse, spreading growth away from the stab line indicated motility, while confined growth along the stab line indicated non-motile bacteria.

5. Indole Test: Isolates were grown in tryptone broth and incubated at 37 °C for 48 hours. Kovac's reagent was added; a red ring at the surface indicated indole production from tryptophan metabolism.
6. Methyl Red (MR) Test: Isolates were inoculated into MR-VP broth and incubated for 48 hours. The addition of methyl red indicator produced a red color in positive cases, showing mixed acid fermentation.
7. Voges–Proskauer (VP) Test: After incubation in MR-VP broth, Barritt's reagents (α -naphthol and KOH) were added. The development of a red color indicated acetoin production, confirming butanediol fermentation.
8. Citrate Utilization Test: Isolates were streaked on Simmons' citrate agar and incubated at 37 °C. A color change from green to blue indicated utilization of citrate as the sole carbon source.
9. Sugar fermentation Tests: Isolates were inoculated into broth media containing different sugars (glucose, lactose, sucrose, maltose, and galactose) with phenol red indicator and Durham tubes. Acid production was indicated by a color change from red to yellow, while gas production was detected by bubbles in the Durham tubes.

2.6 Identification of Fungal Isolates

Fungal identification was based on:

- i. Colony morphology (pigmentation, surface texture, margin characteristics) [16].
- ii. Microscopic examination using Lactophenol Cotton Blue (LPCB) staining to observe spore structures, hyphae, and conidiophores [18].
- iii. Comparison with standard descriptions in fungal identification manuals (Barnett & Hunter's *Illustrated Genera of Imperfect Fungi*) [16,18],

2.6.1 Biochemical Tests for Fungal Isolates

The following assays were performed to characterize fungal isolates from stored maize.

1. Amylase Activity (Starch Hydrolysis Test): Fungal isolates were inoculated on starch agar plates and incubated at 25–28 °C for 3–5 days [18]. After incubation, plates were flooded with iodine solution. Clear zones around colonies indicated starch hydrolysis due to amylase production.
2. Protease Activity (Casein Hydrolysis Test): Isolates were cultured on skim milk agar and incubated at 25–28 °C. The presence of clear zones around colonies confirmed protease activity, indicating the ability to degrade proteins.
3. Lipase Activity (Tributylin Agar Test): Fungal isolates were inoculated on tributyrin agar plates and incubated at 25–28 °C. Formation of clear halos around colonies indicated lipase production and lipid degradation [19].
4. Cellulase Activity (Carboxymethyl Cellulose Agar Test): Isolates were grown on CMC agar plates and incubated for 3–5 days. Plates were flooded with Congo red dye and destained with NaCl solution. Clear zones around colonies indicated cellulase activity.
5. Sugar Fermentation Tests: Fungal isolates were inoculated into broth media containing different sugars

(glucose, sucrose, lactose, maltose, and galactose) with phenol red indicator[18]. Acid production was indicated by a color change from red to yellow, while gas production was detected using Durham tubes.

3.Results

Three bacterial isolates obtained from stored maize samples were subjected to biochemical characterization, Gram staining, and morphological examination. The results revealed distinct profiles that enabled identification at the species level. Table 1 presents the viable plate count of the bacterial cultures. Sample B1 showed consistent bacterial counts with a mean of 1.12×10^7 CFU/ml. Sample B2 had slightly lower individual counts but a higher mean of 6.67×10^7 CFU/ml, indicating greater microbial load. Both samples yielded viable bacterial colonies suitable for further identification and characterization.

Table 1: Bacterial Counts (Viable Plate Count) of the Samples from Stored Maize

Sample	1 st Count	2 nd Count	3 rd Count	Mean Bacterial Count (CFUml ⁻¹)
Sample B1	112	110	113	1.12×10^7
Sample B2	65	67	68	6.67×10^7

Table 2 presents the viable plate count for the fungal cultures. Both samples exhibited viable fungal growth. Sample F1 showed moderate colony counts with a mean of 6.27×10^7 CFU/ml, while Sample F2 had fewer individual counts but a slightly higher mean of 6.67×10^7 CFU/ml. These results indicate fungal presence in stored maize, warranting further biochemical and species-level identification.

Table 2: Fungal Counts (Viable Plant Count) of the Samples from Stored Maize

Sample	1 st Count	2 nd Count	3 rd Count	Mean Fungal Count (CFUml ⁻¹)
Sample F1	60	65	63	$x 10^7$
Sample F2	16	17	18	6.67×10^7

Table 3 presents the Gram stain and morphological features of the bacterial isolates from stored maize. Gram staining and morphological examination revealed distinct features for each isolate. Isolate B10 appeared as Gram-positive cocci arranged in irregular grape-like clusters. Isolate B11 was identified as Gram-negative, short rod-shaped bacilli, usually occurring singly or in pairs. Isolate B12 appeared as Gram-negative slender rods, typically occurring singly and motile with polar flagella.

Table 3: Morphological Features of Bacterial Isolates from the Stored Maize

Isolates	Gram Stain Test	Morphological Features
B10	Gram-positive	Cocci arranged in irregular clusters (grape-like appearance)
B11	Gram-negative	Short rod-shaped bacilli appeared singly and some in pairs
B12	Gram-negative	Slender rod-shaped bacilli appeared singly

Table 4 presents the biochemical characterization of bacterial isolates from the stored maize. Biochemical characterization of three bacterial isolates, B10, B11, and B12, revealed distinct profiles. All three isolates tested positive for catalase and methyl-red, while B11 and B12 showed negative for oxidase and Voges-Proskauer activity. B10 was coagulase-positive and non-motile, with acid production from glucose, lactose, and sucrose, and was identified as *Staphylococcus aureus*. B11 exhibited motility, indole positivity, citrate utilization, and gas production from glucose and lactose, consistent with *Escherichia coli*. B12 shared similar traits with B11 but could not ferment sucrose, maltose, and galactose, and was confirmed as *Pseudomonas aeruginosa* based on its non-fermentative profile and biochemical behavior. These identifications support the presence of both enteric and environmental bacteria in stored maize samples.

Table 4: Biochemical characterization of Bacterial Isolates from Stored Maize

Test	B10	B11	B12
Catalase	+	+	+
Coagulase	+	-	-
Oxidase	-	-	+
Motility	-	+	+
Indole	-	+	-
Methyl-Red	+	+	-
Voges-Proskauer	+	-	-
Citrate Utilization	-	-	+
Glucose	+ /A	+ /AG	-
Lactose	+ /A	+ /AG	+
Sucrose	+ /AG	+	-
Maltose	+	+	+
Galactose	+	+	+
Probable bacteria	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>

+: positive, -: negative, +/A: Acidic only +/AG: Acidic and gaseous

Table 5 and 6 present the morphological and biochemical characterization of the fungal isolates from stored maize. Biochemical and morphological analyses were conducted on three fungal isolates labeled F10, F11, and F12. Isolate F10 exhibited strong enzymatic activity, including positive reactions for amylase, protease, lipase, and cellulase, and was utilized glucose, lactose, sucrose, maltose, and galactose. Morphologically, it presented as fast-growing colonies with a velvety texture and bluish-green pigmentation, consistent with *Aspergillus fumigatus*. Isolate F11 showed positive activity for amylase and protease, limited lipase and cellulase activity, and fermented glucose and sucrose but not lactose or maltose. Its dark, woolly colonies and biserial conidial heads confirmed its identity as *Aspergillus niger*. Isolate F12 demonstrated protease and lipase activity, with limited sugar fermentation, and was identified as *Rhizopus microsporus/stolonifer* based on its rapid growth, cottony white colonies, and characteristic sporangial structures. These findings confirm the presence of both *Aspergillus* and *Rhizopus* species as fungal contaminants in stored maize.

Table 5: Microscopic Morphological Features from the Stored Maize

Isolates	Colonial Features	Morphological Features
F10	White, turned greenish-yellow and pale to tan on the underside	Short conidiophores, small and smooth conidia, and columnar vesicles
F11	Black and powdery, and pale yellow on the underside	Long and smooth conidiophores, globose vesicles, and rough and dark conidia
F12	White cottony turned gray to black, and pale to colorless on the underside	Hyphae- Broad coenocytic (aseptate) and hyaline Sporangiospores- smooth and oval to round in size Rhizoids- root-like structures

Table 6: Biochemical characterization of Fungal Isolates from Stored Maize

Activity	F10	F11	F12
Amylase	+	+	+
Protease	+	+	+
Lipase	+	±	+
Cellulase	±	+	±
Glucose	+	+	+
Lactose	±	-	-
Sucrose	+	+	+
Maltose	+	+	+
Galactose	±	+	±
Probable Species	<i>Aspergillus species</i>	<i>Aspergillus species</i>	<i>Rhizopus species</i>
Probable fungi	<i>A. fumigatus</i>	<i>A. niger</i>	<i>R. microspores/stolonifer</i>

+: positive, -: negative

Notes: “±” indicates variable activity commonly reported across strains

4. Discussion

The microbiological analysis of stored maize revealed the presence of both bacterial and fungal contaminants, each with distinct implications for food quality and safety. Bacterial counts ranged between 1.12×10^7 and 6.67×10^7 CFU/ml. In contrast, fungal counts were similarly high, between 6.27×10^7 and 6.67×10^7 CFU/ml. These values indicate substantial microbial loads, reflecting poor storage conditions and the susceptibility of maize to contamination during handling and preservation.

Stored maize contaminated with microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Rhizopus microsporus* presents significant implications for both maize quality and food safety. These organisms are capable of altering the physical, nutritional, and

sensory properties of maize during storage. Fungi like *Aspergillus* and *Rhizopus* secrete hydrolytic enzymes, including amylase, protease, lipase, and cellulase, which degrade starch, proteins, and lipids[20, 21]. This enzymatic activity leads to loss of texture, discoloration, off-flavors, and reduced nutritional value, thereby shortening the shelf life of maize and making it unsuitable for consumption or trade[18, 20].

Biochemical and morphological characterization identified three bacterial isolates: *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The presence of *S. aureus* is significant because of its ability to produce enterotoxins that cause food poisoning [14], while *E. coli* is a well-known indicator of fecal contamination and a potential source of gastrointestinal infections[14, 22]. *P. aeruginosa*, though primarily environmental, is an opportunistic pathogen capable of causing infections in immunocompromised individuals [23]. Together, these bacteria highlight both hygiene lapses and environmental exposure as contributing factors to maize contamination.

The findings of this study, which identified *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Rhizopus microsporus/stolonifer* in stored maize, align closely with previous research on microbial contamination of maize and maize-derived products. Osuntokun and his colleagues similarly reported the presence of *E. coli*, *Salmonella enterica*, and *S. aureus* in infected maize using 16S rRNA sequencing, confirming that pathogenic bacteria are common contaminants of stored grains[14]. Their work also highlighted the limitations of conventional methods, as molecular characterization provided more precise identification of bacterial species. Orogu and his colleagues extended this understanding by demonstrating that stored corn harbor spoilage bacteria such as *Pseudomonas*, *Bacillus*, and *Lactobacillus*, which persist even after heat treatment, emphasizing that cooking does not guarantee complete elimination of microbial hazards [17]. This supports the current study's observation of *Pseudomonas aeruginosa* in maize.

Additionally, fungi are notorious for their spoilage potential and, in the case of *Aspergillus* species, their ability to produce mycotoxins such as aflatoxins and ochratoxins [6]. Such toxins are stable compounds that persist even after cooking, posing long-term health risks, including liver damage and carcinogenic effects[6]. *Rhizopus* species, while primarily associated with spoilage, can also cause opportunistic infections[24]. Their rapid growth and enzymatic activity contribute to the deterioration of maize quality, reducing its nutritional value and shelf life.

Fungal contamination observed in this study also resonates with earlier reports. Popoola and Okungbowa identified *Aspergillus*, *Trichoderma*, *Penicillium*, and *Rhizopus* species in stored corn, noting their enzymatic activities (protease, amylase, lipase) as key contributors to spoilage and postharvest losses[18]. The detection of *Aspergillus fumigatus*, *A. niger*, and *Rhizopus microsporus* in the present work confirms the spoilage potential of these fungi and their role in reducing maize quality. Abe and his colleagues further demonstrated the diversity of filamentous fungi in maize grains with rot symptoms, many of which are prolific producers of hydrolytic enzymes[16]. Their findings highlight the enzymatic versatility of maize-associated fungi, which mirrors the enzymatic activity observed in the isolates of this study. The overlap in microbial species across different investigations demonstrates the consistency of contamination patterns and the persistence of these organisms in maize storage systems. This convergence of evidence validates the urgent need for improved postharvest practices, including moisture control, hygienic handling, and microbial monitoring, to safeguard maize quality and protect public health.

5. Conclusion

This study demonstrated that stored maize is susceptible to microbial contamination, with both bacterial and fungal isolates identified. The bacterial contaminants included *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, while the fungal isolates comprised *Aspergillus fumigatus*, *Aspergillus niger*, and *Rhizopus microsporus/stolonifer*. High microbial counts confirmed poor storage conditions and highlighted the dual threat of spoilage and pathogenicity. The presence of toxin-producing fungi such as *Aspergillus* species and pathogenic bacteria such as *E. coli* and *S. aureus* indicates the serious food safety risks associated with contaminated maize. Conclusively, the evidence confirms that microbial contamination of maize is a persistent challenge with implications for public health, food security, and economic stability.

6. Recommendations

- Improved storage practices: Maize should be stored under dry, well-ventilated conditions with moisture levels below 13% to inhibit microbial growth.
- Routine microbial monitoring: Regular screening for bacterial and fungal contaminants, including mycotoxin analysis, should be incorporated into postharvest management.
- Hygienic handling: Farmers, traders, and consumers should adopt strict hygiene practices during harvesting, processing, and storage to minimize contamination.
- Use of biocontrol agents: Beneficial microorganisms such as *Lactobacillus* species or non-toxin-producing fungi can be explored as biological control agents to suppress pathogenic strains.
- Public awareness: Education campaigns should inform communities about the risks of consuming contaminated maize and the importance of safe storage practices.

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