Faculty of Natural and Applied Sciences Journal of Applied Biological Sciences
Print ISSN: 3027-0685, e-ISSN: 3043-6494
www.fnasjournals.com
Volume 2; Issue 2; March 2025; Page No. 49-55.

Isolation and Identification of Bacterial Contaminants from Spoiled Fruits Sold at Agajia Market, Bonny Island

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Abstract
Microscopic organisms, specifically bacteria, play a crucial role in fruit spoilage. These bacteria thrive under favorable temperature conditions when food and water are available, leading to changes in the appearance, color, and odor of the fruit. This research aimed to evaluate the various bacterial species present in three types of spoiled fruits: pineapple (*Ananas comosus*), banana (*Musa paradisiaca L.*), and apple (*Malus pumila*). Four bacterial species— *Escherichia coli*, *Klebsiella sp*., *Bacillus sp*., and *Staphylococcus sp*.—were successfully isolated using the serial dilution and agar plating techniques. Gram staining analysis confirmed that *Bacillus sp*. and *Staphylococcus sp*., isolated from banana samples, were Gram-positive bacteria. Similarly, *Escherichia coli* found in apples was also Gram-positive, exhibiting a long rod shape, cocci in clusters, and a short rod morphology, respectively. Meanwhile, *Escherichia coli* and *Klebsiella sp*. from pineapple samples were identified as Gram-negative bacteria. Microscopic observations revealed that *Escherichia coli* and *Klebsiella sp*. had short rod-shaped colonies. Biochemical analysis demonstrated that the two most prevalent bacterial species in the spoiled fruits were *Escherichia sp.* and *Klebsiella sp*. To improve fruit preservation methods, further advancements in conservation strategies are recommended. Additionally, government regulatory bodies should raise awareness among farmers and vendors to enhance the quality of fresh produce.

Keywords: Bacterial Contamination, Spoiled Fruits, Food Safety, Market Produce, Microbial Analysis

Introduction

Fruits are rich sources of essential vitamins, minerals, and dietary fiber, making them crucial for maintaining overall health. They also contain significant amounts of antioxidants, which help protect the body against various diseases. However, one of the major challenges associated with fruits is their short shelf life due to their susceptibility to microbial contamination. Bacteria, often introduced through exposure to soil, dust, and water, play a significant role in fruit spoilage, emphasizing the need for proper storage and preservation techniques to extend their usability and prevent waste. Several countries engage in large-scale fruit production, with Nigeria ranking among the leading agricultural nations in Africa. Despite this high level of production, a substantial portion of harvested fruits is lost due to poor handling, inadequate transportation, and insufficient storage facilities (Zubbair, 2009; Chukwuka et al., 2010; Barth et al., 2013). Over the years, fruit consumption in Nigeria has increased by more than 30%, primarily due to heightened awareness of the benefits of a healthy diet. However, approximately 20% of the fruits produced annually are lost to spoilage. The Department of Statistics (DOS, 2017) reported that around 18.9 billion pounds of fresh fruit were wasted each year, representing 19.6% of the country's total edible food losses during the reviewed period. Fruits provide an ideal environment for microbial growth, particularly bacteria, due to their rich content of sugars, minerals, vitamins, and amino acids (Bhale, 2011). Food spoilage occurs when changes in composition render food unsafe or unsuitable for human consumption (Akinmusire, 2011). Bacterial spoilage of fruits typically begins with the breakdown of pectins, leading to tissue softening and decay, which manifests as a slimy texture. The metabolism of starches and sugars by bacteria produces undesirable odours and flavours, along with substances such as lactic acid and ethanol (Rawat, 2015). Some bacteria can even invade intact fruit tissues, resulting in lesions (Clauss et al., 1986).

Microbial contamination of fruits can occur at various stages, including cultivation, harvesting, handling, transportation, storage, and marketing. Additionally, improper hygiene practices after purchase can contribute to contamination. Spoiled fruits not only lose their visual appeal but can also pose health risks to consumers.

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Research has identified various bacterial species in decayed fruits, including Pseudomonas, Erwinia, Xanthomonas, Enterobacter, Flavobacterium, Chromobacterium, Lactobacillus, Bacillus, and Clostridium. Fruits contribute significantly to human nutrition by supplying essential nutrients, including vitamins, minerals, and dietary fiber. However, post-harvest preservation remains a major challenge. This study seeks to identify bacterial species responsible for the deterioration of three economically important local fruits. Pathogenic bacteria can infiltrate fruits through openings such as cuts and wounds, where they continue to grow, increasing the risk of foodborne illnesses (FDA, 1999). For instance, Salmonella species have been found to multiply rapidly on watermelon stored at room temperature, with minimal reduction even under refrigeration (FDA, 1999).

Statement of the Problem

Food products left exposed in open environments are highly susceptible to bacterial contamination. Concerns have arisen regarding the safety of fruits sold at Akiama Market in Bonny, following reports of illnesses among consumers (Ikechukwu et al., 2023). The fruit vending business in Bonny Island serves as a vital source of employment and contributes to the informal economy. It also plays a role in food security by providing affordable fruit options to residents. However, there are no documented studies evaluating the effectiveness of measures taken to ensure the safety of fruits sold in Bonny. Given the potential health risks associated with fruit contamination, it is necessary to investigate the situation, particularly in high-risk areas such as Bonny Island. This research aims to isolate and identify bacterial species present in fruits sold at Akiama Market in Bonny Local Government Area, Rivers State, Nigeria.

Aim and Objectives of the Study

The aim of the study was to evaluate the various bacterial species on spoiled fruits sold in Akiama Market in Bonny Local Government Area of Rivers State, Nigeria with the following objectives as;

1. To isolate bacteria from Spoiled fruit sold in Akiama market in Bonny Local Government Area.
2. To characterize and identify the isolates, using colonial morphological characteristics.
3. To characterize and identify the isolates, using biochemical test and gram staining.
4. To make observations and recommendations to Bonny Local Government regulatory bodies to provide guiding rules to fruits Vendors and improved technology based on preservative methods to enhance the quality of fruits from farmer.

Methods and Materials

Study Area

The study was conducted at Akiama Market in Bonny Local Government Area of Rivers State. Bonny, also known as Ibani, is an island settlement and Local Government Area in southern Nigeria, located along the Bight of Bonny. The island is situated near Port Harcourt, with maritime transport serving as the primary means of travel to and from the area. According to the 2006 census, Bonny had an estimated population of 214,983. The Local Government Area spans approximately 645.60 km² and lies between latitudes 4°26′N to 5°02′N and longitudes 6^0 56'E to 7°10′E. The region comprises two major urban centers, Bonny and Finima, with primary economic activities including fishing, commercial trade, and employment in both civil and public service sectors.

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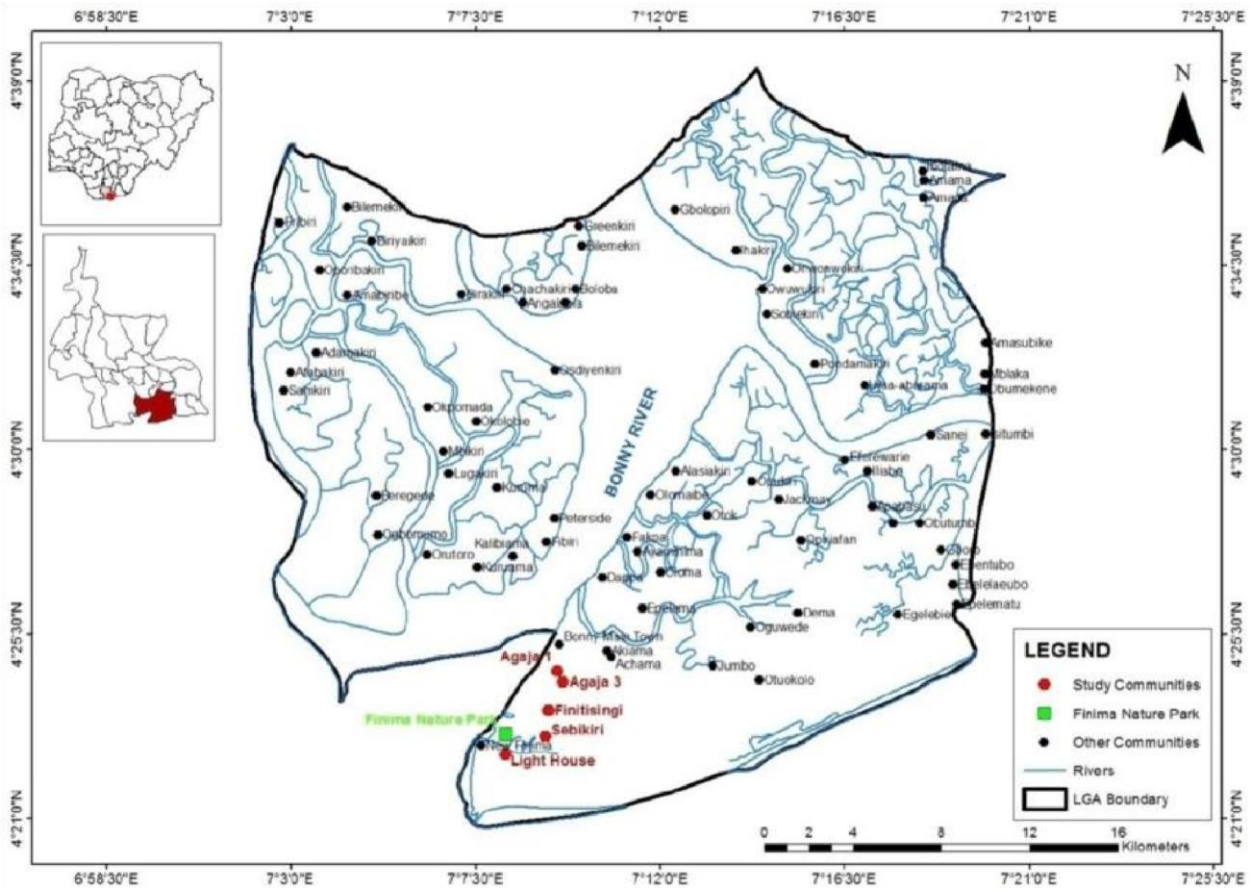

Fig.1 Map showing Bonny Island
Source: Digitized from the original Map, Ministry of lands and survey, Port Harcourt

Sample Collection
Three types of overripe and decayed fruits—bananas, apples, and pineapples—were obtained from local vendors at Akiama Market, Bonny Island. Each sample was securely placed in sterile polythene bags and transported in an ice-packed cooler to a microbiology laboratory for further analysis.

Bacterial Isolation
Using the serial dilution method, life forms were separated from weak common things.
The sullied standard thing tests were mixed with refined water to make a suspension, which was at that point weakened in a course of development from 10^1 to 10^6. A 100 µL aliquot from each weakening was spread onto pre-labeled supplement agar plates to back bacterial development. Contrasts in colony morphology were watched to consider bacterial contrasts. The bacterial limits were in this way sub-cultured and put misplaced on supplement agar inclines at 4°C for advancement examination.

Characterization and Identification of Bacterial Isolates
To obtain pure strains, distinct bacterial colonies from supplement agar and MacConkey agar plates were carefully selected and subcultured multiple times. The confines were brought forward at 37°C for 24 hours. Once cleaned, they were guaranteed on supplement agar inclines at 4°C for advance examination. Characterization and recognizable statement were conducted taking after standard microbiological techniques, checking Gram recoloring and biochemical tests such as catalase, indole, methyl blue, Voges-Proskauer, citrate utilization, and coagulase tests.

Gram Staining Technique
Gram staining was used to classify small life forms based on their ability to retain crytal violet recolor following liquor decolorization. The handle involved:

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Applying a slant bacterial spread to a glass slide that has been degreased. • Heat-fixing the spread by briefly passing it through a flame.

Washing with clean water and recoloring with crystal violet for one less than anticipated

Applying iodine course of activity for one more humble than anticipated, taken after by rinsing.

Flushing once more after decolorizing with 95% ethanol until the excess recolour is gone. Counterstaining with safranin for one scaled down, taken after by washing and air-drying. A few times recently during a minor examination at the 100x update, a drop of sprinkling oil was mentioned. Little living creatures holding the gem violet recolor showed up purple or basic blue (Gram-positive), in appear despise toward of the truth that those losing the recolor and taking up safranin showed up pink or blue (Gram negative)

Indole Test

The ability of a bacterium to convert tryptophan into indole was determined by this test. Peptone water was used to immunize a bacterial colony, which was then brooded at 37°C for 18–24 hours. The development of a primary reddish color indicated a positive result after carefully shaking 0.5 mL of Kovac's reagent, in contrast to the appearance of a pale yellow color indicating a negative result.

Methyl Red Test

The methyl blushing test was used to determine whether mixed dangerous change had occurred in small life forms. In a test tube, five drops of the methyl ruddy marker were added to a 4 mL bacterial culture in peptone water. The best result was a blushing ring, while a pale yellow or no color change resulted in a negative result.

Voges-Proskauer Test

The purpose of this test was to distinguish acetoin period from glucose production. A 4 mL
bacterial culture in peptone water was blended with 0.6 mL (six drops) of Barritt's reagent A and 0.2ml (four drops) of Barrit's reagent B. In contrast to the absence of color change, the appearance of getting to be flushed color internal parts after 15 to 20 minutes produced a positive result.

Citrate Utilization Test

The citrate utilization test chosen whether little living creatures may utilize sodium citrate as their sole carbon source. The bacterial drag back was applied to each Simmons citrate agar plate, and it was brooded at 35°C for 24 to 48 hours. Despite the fact that a green color produced a negative result, a crucial blue color revealed a positive outcome.

Results

Morphological Characteristics of Bacterial Isolates

Table 1 presents the morphological features of bacterial isolates recovered from the deteriorated fruit samples. The findings confirmed that all analyzed fruit samples contained one or more microbial species, exhibiting distinct structural and growth variations.

Biochemical Characterization and Identification of Bacterial Isolates

Table 2 outlines the results of the biochemical assays and Gram staining conducted on the bacterial isolates. The analysis identified bacterial species such as Escherichia coli, Klebsiella sp., Bacillus sp., and Staphylococcus sp. in the decayed fruit samples collected from Akiama Market, Bonny Island.

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Table 1. Morphological characteristics of bacteria isolated from spoiled fruits in Akiama market

S/N	Fruits	Isolate	Colonial morphology	Gram reaction	Shape and arrangement
1	Pineapple	*Escherichia coli*	White, opaque, large, smooth, flat, moist	-	Short rod in scattered arrangement
2	Pineapple	*Klebsiella sp.*	Light pinkish, translucent, and raise growth	-	Short rod in scattered arrangement
3	Banana	*Bacillus sp.*	White, attached spreading with crenate margin, dry	+	Long rods with round edges in scattered arrangement
4	Banana	*Staphylococcus sp.*	Golden yellow, large, opaque, convex smooth and shiny.	+	Cocci in bunches
5	Apple	*Escherichia coli*	White colony	+	Short rod in scattered arrangement
6	Apple	*Klebsiella sp.*	Light pinkish, translucent, and raise growth	-	Short rod in scattered arrangement

+ = positive, - = negative

Table2: Characterization and Identification of bacteria isolates using biochemical tests and Gram staining.

Bacteria isolates	Gram reaction	Biochemical tests			
	Gram staining	Indole test	Methyl red test	Voges proskauer test	Citrate test
Escherichia coli	Gram –ve	-	+	-	-
Klebsiella sp.	Gram –ve	-	-	+	+
Bacillus sp.	Gram +ve	-	-	+	+
Staphylococcus sp.	Gram +ve	-	+	+	-
Escherichia coli	Gram +ve	-	+	-	-
Klebsiella sp.	Gram –ve	-	-	+	+

- = Negative, + = positive, -ve = negative and +ve = positive

Discussion

Bacterial Contamination and Fruit Spoilage: An Investigative Study

Fruits serve as a natural source of vital nutrients, including vitamins, minerals, carbohydrates, and other essential compounds. However, their high moisture content makes them highly prone to bacterial contamination, which accelerates spoilage. This research successfully identified four bacterial species from three types of decayed fruits—pineapple, banana, and apple—sourced from Akiama Market in Bonny Island, Rivers State, Nigeria.

Classification of Bacteria Using Gram Staining

Bacteria can be classified based on the Gram staining technique, as described by Goldman and Green (2006). In this study, three Gram-positive bacterial species were detected in decayed banana and apple samples, while three

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Gram-negative species were identified in spoiled pineapple and apple samples. Gram-positive bacteria possess a thick peptidoglycan layer that retains the crystal violet-iodine complex, giving them a purple coloration. In contrast, Gram-negative bacteria have a relatively thin peptidoglycan layer and an outer lipid membrane, which allows the crystal violet stain to be removed by alcohol, causing them to appear pink.

Morphological Examination of Bacterial Isolates

A detailed morphological analysis was conducted to assess the physical traits of the bacteria, such as their shape, texture, and coloration. The primary aim was to group bacteria with similar structural features. Isolates were classified within the same genus if their morphological characteristics matched descriptions from established bacteriological studies. The findings revealed significant differences in colony growth patterns and pigmentation across bacterial species. Table 1 summarizes the morphological traits of the identified bacteria. *Escherichia coli* and *Klebsiella sp.* were isolated from spoiled pineapple and apple samples. Microscopic observation showed that all bacterial isolates had a short rod shape and appeared scattered. Bacillus sp. displayed a long rod-shaped structure, whereas *Staphylococcus sp*. had a cocci-shaped appearance and formed clusters.

Biochemical Identification Using IMViC Tests

IMViC tests were utilized to distinguish coliform bacteria. As indicated in Table 2, all bacterial isolates tested negative for the indole test. According to Goldman and Green (2006), this test differentiates bacteria capable of producing the enzyme tryptophanase, which breaks down tryptophan into indole.

The Methyl Red test

Escherichia coli and *Staphylococcus sp*. tested positive, turning the medium red, indicating acid production with a pH below 4.4. In contrast, Escherichia coli showed a negative result in the Voges-Proskauer test, suggesting its inability to produce acetoin from glucose metabolism. However, *Bacillus sp*. and *Klebsiella sp*. tested positive in the citrate test, confirming their capability to use citrate as a sole source of carbon and energy. Based on their biochemical properties, the bacterial species isolated from the decayed fruit samples were *Escherichia coli*, *Klebsiella sp*., *Bacillus sp*., and *Staphylococcus sp*. Previous studies have similarly reported the presence of Escherichia coli and Bacillus sp. in spoiled papaya (Ismail & Zhang, 2004). Additionally, *Klebsiella sp*. and *Staphylococcus sp*. were detected in a study analyzing 30 samples of decayed fruits (Kumar et al., 2011).

Conclusion

This study successfully identified six bacterial species from three types of decayed fruit samples. Further research is needed to determine the specific role of these bacteria in fruit spoilage. The high bacterial load observed in the analyzed fruits underscores the importance of educating the public about the health risks associated with poor handling of fresh produce. To preserve fruit quality, advanced preservation methods should be adopted. Additionally, regulatory bodies must implement microbiological guidelines to ensure that farmers, vendors, and hawkers adhere to proper handling and distribution practices.

Recommendations

The study identified several key factors contributing to fruit contamination, including inadequate hygiene among vendors, poor handwashing habits, the use of unsanitary utensils, and improper storage methods. To minimize bacterial contamination, the following are recommended;

1. Fruits should be handled with properly washed and sanitized hands and utensils.
2. If fruits are not consumed immediately, they should be stored in refrigerated conditions.
3. These findings emphasize the need for food safety education and the promotion of hygienic handling practices to prevent bacterial spoilage and the spread of harmful microorganisms.
4. Additionally, efforts should be made to develop better preservation techniques to prolong fruit shelf life.
5. Vendors selling fruits on the streets should receive adequate training on food safety and hygiene.
6. Government agencies should establish and enforce strict regulations to ensure that vendors adhere to proper handling and storage guidelines.

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