ISOLATION AND CHARACTERIZATION OF MICROBES ASSOCIATED WITH THE DETERIORATION OF BANANA FRUITS

PATRICK OLORUNFEMI OLADELE; IFEOMA S. ASOGWA; AHMED E.; MOSES ANTHONY; & ANTHONY C. AGBO

Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Bingham University, Karu, Abuja, Nigeria.

sandra-ifeoma.asogwa@binghamuni.edu.ng

ABSTRACT

Banana fruits are staple food source for millions of people worldwide which has led to an increased global demand for the fruit. However, they are susceptible to various forms of deterioration which is often attributed to the activity of microorganisms, including bacteria, fungi and yeasts, which can cause various types of spoilage, such as discoloration, texture changes, off flavors, etc and can lead to post-harvest losses. This study was carried out to isolate and identify microorganism associated with the deterioration of bananas fruits. A total of 4 samples were examined for fungal and bacteria species by serial dilution, inoculation and cultured on Nutrient agar and Sabouraud Dextrose Agar respectively. Both cultured plates were incubated at 37^0c for 24 hours and at 28 ±1°C for five days, and sub cultured respectively. Macroscopic and microscopic examination of the fungal species was conducted, and morphological features were studied and used for fungal identification by comparison with a standard fungal identification guide. Morphological examination, Gram staining and biochemical test were also conducted to identify bacteria species. The bacteria count ranged from 4.5 x 105 to 1.21 x 106 CFU/ml indicating higher amounts of bacteria population in the spoilt samples. *Fusarium spp, Rhizopus spp* and *Candida spp* were the most isolated fungi from fresh banana fruits with an occurrence of (2)25% while *Aspergillus flavus* and *Candida spp* were the least isolated with an occurrence of (1)12.5%. *Aspergillus fumigatus*, *Rhizopus spp, Mucor spp* and *Candida spp* were the most isolated fungi from spoilt banana fruits with an occurrence of (2)20% while *Fusarium spp* and *Aspergillus flavus* was the least isolated with an occurrence of (1)10%. *Staphylococcus aureus* and *Streptococcus pyogenes* were the least isolated bacteria from fresh banana fruits with an occurrence of (1) 25% while *Escherichia coli* was the most isolated with an occurrence of (2) 50%. Staphylococcus aureus and *Streptococcus pyogenes* were the least isolated bacteria from spoilt banana fruits with an occurrence of (1) 25% while *Escherichia coli* was the most isolated with an occurrence of (2) 50%. The presence of the isolates may be due to the careless handling and storage conditions of banana fruits which would have bruised or cut the

banana peel. Another factor that could lead to the its spoilage was improper hygienic practices from the point of harvesting, transportation and storage by handlers. The high moisture content of banana fruit makes it highly perishable and contributes to its low shelf life, as it supports the growth of microbes.

KEYWORDS: Banana, Deterioration, Moisture Content, Careless Handling, Microscopy, Macroscopic.

INTRODUCTION

A fruit is the edible part of a mature ovary of a flowering plant. It is usually eaten raw. When mature, they may be either fleshy or dry. Fleshy fruit are further classified into berry (orange, tomato, pineapple, pawpaw, and banana), drupes (plume, coconut, almond, cherry) and pomes such as apple and pear. The dry fruits, unlike the fleshy fruits which have unlayered pericarp, are classified into dehiscent (pod, follicle and capsule) and indehiscent fruits like achene, samara, cashew etc. (Jay, 2000). It is well known that fruits constitute commercially and nutritionally important indispensable food commodity. Fruits play an important role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help to keep a good and normal health. Fruits are widely distributed in nature. Banana *(Musa paradisiaca L.)* is an edible fruit, botanically a berry, produced by several kinds of large herbaceous flowering plants in the genus Musa. The fruit which grows in clusters is variable in size, colour and firmness, but is usually elongated and curved, with soft pulpy flesh in starch covered with a rind which may be green, yellow, and red, when ripe. There are about 1000 varieties of bananas in the world. In some countries, bananas used for cooking may be called plantains (Chibuzor *et al.,* 2019.)

In the developing world, banana is a major staple crop of considerable importance. Although banana fruits are highly demanded as nutritious and economically important fruits, they experience a different marketing problem (El-Naby, 2010). One of the limiting factors that influence the fruit's economic value is the relatively short shelf life period caused by microbial attacks. Fruits contain high levels of sugars and nutrient elements and their low pH values make them susceptible to fungal decay (Singh & Sharma, 2007). Most producers and consumer of banana determines the quality of the banana by the colour of the peels. The colour of the fruit could serve as an indication of the state of deterioration and/or disease. Banana fruits are attacked by various pathogenic and non-pathogenic bacteria, viruses, fungi, helminthes and protozoa, during their growing seasons, harvesting, handling, transportation and post-harvest storage/marketing conditions, or after purchasing by the consumer (Argudn, *et al,* 2010). Toxigenic fungi have been isolated from spoiling fruits. During refrigeration, some moulds may

produce mycotoxins. Pathogenic fungi, on the other hand, could cause infections or allergies (Battilani *et al,* 2016).

Global production of banana has already increased by approximately 24% from 97 million metric tons in 2008 to nearly 125 million tons in 2021 (Shahbandeh, 2023). This increment has been gradual and hence the increase in exports is at pace with the growth of bananas worldwide (Food and Agriculture Organization, 2020). The banana producing sector has also been concerned with Microorganisms, natural catastrophes such as drought, floods etc. which makes it more challenging, risky to grow bananas and has naturally affected the Global fruit production.

It is estimated that about 20%−25% of the harvested fruits are decayed by pathogens during post-harvest handling, even in developed countries. In developing countries, post-harvest losses are often more severe due to inadequate storage and transportation facilities, and everyday 1.6 million bananas are thrown away in developing countries (Al-Shannaq, *et al* 2017).

Diseases caused by eating contaminated fruits and vegetables are known as food-borne disease. Food borne diseases can affect a population by lowering social economic status, loss of productivity, increased healthcare costs, depression, outbreak of epidemic diseases, etc. (Health and Safety 2022).

Increased demand for the production and consumption of banana fruits has led to high loss of the fruits due to microbial spoilage. Food borne diseases continue to be a common and serious threat to public health all over the world and these diseases are a major cause of morbidity. Outbreaks of human infections due to the consumption of raw fruits and vegetables have occurred with increased frequency during the past decade (Etewa *et al*, 2017, Bekele and Shumbej, 2019).

Over the years, there has been an increase in the need to identify and isolate the microorganisms associated with the spoilage as a way of finding a means of controlling it (Ankar-Brewoo, 2018, Acheampong, 2015).

This study aimed on microorganisms associated with spoilage in fresh and spoilt banana. Thus this provided a data base on microbial spoilage of banana. Understanding the microbial agents involved in banana fruit spoilage is crucial for developing effective preservation and management strategies to minimize post-harvest losses, extend shelf-life, improve quality and ensure food security.

MATERIAL AND METHOD

MEDIA

The media used to carry-out this research were: Nutrient agar and Sabouraud dextrose agar (Titan Biotech Ltd, India.

EQUIPMENT

The equipment used includes: Incubator, Digital Weighing scale, Autoclave, Water distiller Test tubes, Conical flask, Electronic microscope, Inoculating loop.

REAGENT

The reagents were: Kovacs reagent, Peptone water (Himed labs,Mumbai India), Hydrogen peroxide. Gram stain.

THE STUDY AREA

The study area is at Mararaba Market, in Nasarawa State. Mararaba market is a large and crowded market with lots of stuffs to buy and sell. It has a great location for a market. Nasarawa state is in the North central region of Nigeria, with Latitude: 8.4991^0N and Longitude: 8.5156^0 E, bordered to the east by states of Taraba and Plateau, to the North by Kaduna state, to the south by the states of Kogi and Benue, and to the West by the Federal Capital Territory (National Bureau of Statistics, 2006).

SAMPLE COLLECTION

Four (N=4) samples of fresh and spoilt banana were collected from different banana vendors in Nasarawa metropolis. 4 samples (2 fresh and 2 spoilt) were collected from different vendors each in Mararaba market in clean polythene bags separated and taken to the Department of Pharmaceutical Micro-biology laboratory, Bingham University for analysis.

SAMPLE PREPARATION

1g of the banana pulp was weighed using a digital weighing scale and macerated in a mortar and homogenized in 9ml of sterile distilled water. A tenfold serial dilution of the homogenates were prepared using sterile pipettes.

MEDIA PREPARATION

The media used for the experiment were Nutrient agar and Sabouraud dextrose agar for isolation of bacteria and fungi respectively. The media were prepared using manufacturers instruction and sterilized using an autoclave at 121°C for 15 minutes.

ISOLATION OF BACTERIA AND FUNGAL ISOLATES FROM FRESH AND SPOILT SAMPLES

The pour plate method of Harigan and McCane (1990) described by Mbajiuka *et al.,* (2014) was used. From the tenfold dilution of the homogenates, 1ml of the diluted homogenates was aseptically inoculated onto Nutrient agar plates. The plates were allowed to solidify and then

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incubated at 37^0C for 24hrs. Plates were examined for bacterial growth after 24hours and colonies counted using a colony counter and enumerated as cfu/g. Colonies from the primary plates were aseptically picked with a sterile wire loop and transferred onto freshly prepared sterile nutrient agar plate, with a streaking technique such that discrete colonies appear at the ends of streaked lines after incubation. The sub-cultured plates were incubated at 37°C for 24 hours. The same method was carried out for the isolation of fungal isolates but a different media (Sabouraud dextrose agar) was used.

CHARACTERIZATION AND IDENTIFICATION OF ISOLATES

All bacterial isolates and fungal isolates were characterized and identified considering their cultural, morphological, microscopic examination and biochemical characteristics following the methods described by Holt *et al.,* (1994). Gram staining test and Biochemical test was also conducted. The Biochemical test include the following: Catalase test, Coagulase test and Indole test.

GRAM STAIN REACTION

A sterile wire loop was used to obtain a colony of the isolate on the plate and emulsified on a clean glass slide, it was smeared and air dried completely. The fixed smear was covered with crystal violet stains for 30 seconds. The stain was washed with clean water, covered again with lugos iodine for 30 seconds and rinsed with clean water. The smear was then decolorized with acetone and flushed with clean water; the smear was then covered with natural red stain for 2 minutes and rinsed with clean water. The back of the slide was wiped clean and allowed to air dry after which the smear was examined microscopically. First with the x40 objective to check the stain to see the distribution of the staining reagent and then with the oil immersion x10 objective to examine the bacteria.

CATALASE TEST

A smear of a small portion of a colony under test was placed into a tube containing about 2 ml of hydrogen peroxide. Catalase positive strains caused effervescence (air bubbles) while catalase negative does not. The presence of bubbles indicated *Staphylocoocus spp* was present.

COAGULASE TEST

A drop of normal saline was placed on a clean slide. About one or two colonies of the test organism were picked with a sterile loop and emulsified in the drop of saline to form a smooth milky suspension. The inoculating wire-loop was dropped into undiluted plasma obtained by centrifuging human blood Coarse clumping become visible to the naked eye within 5-10 seconds indicated a positive result while no reaction indicated negative result.

INDOLE TEST

The peptone water medium was inoculated and incubated for 48 hours at 37°C. After 48 hours of incubation, 3 drops of Kovac's reagent was added and was shook very well and allowed to stay for 15 minutes (in each tube). The red ring on the surface of the peptone water indicates positive result, while yellow ring indicates negative result.

MICROSCOPIC EXAMINATION OF FUNGAL ISOLATES

Lacto phenol cotton blue was dropped on a glass slide and small portion of fungal colony from the sub-cultured plates was taken using a sterile inoculating needle and transferred to a glass slide, it was then emulsified with a sterile needle and then covered with a cover slip gently to avoid air bubbles. Observation under the objective lens was carried out, the observation include, searching for different features of fungi including, the hyphae, conidia, sporangiosphore (reproductive structure) and identification was carried out microscopically by examining the colony and comparing using a fungi identification chart (Adebayo-Tayo *et al*., 2012; Onuorah *et al*., 2015)

RESULTS

Six different types of fungi isolates was obtained from this study. Fungal isolates examined include; *Fusarium spp, Aspergillus flavus, Aspergillus fumigatus, Rhizopus spp*. The yeast isolated was *Candida spp. Mucor spp* was also isolated.Three strains of bacteria identified by their Gram stain reaction and biochemical characteristics were *Staphylococcus aureus*, *Streptococcus species* and *Escherichia coli*.

Fusarium spp, Rhizopus sppand Candida spp were the most isolated from fresh banana fruits with an occurrence of (2)25% while *Aspergillus flavus* and *Candida spp* were the least isolated with an occurrence of (1)12.5% **(Table 4).**

Aspergillus fumigatus, Rhizopus spp, *Mucor spp and Candida spp* were the most isolated from spoilt banana fruits with an occurrence of (2)20% while *Fusarium spp* and *Aspergillus flavus* was the least isolated with an occurrence of (1)10% **(Table 5).**.

Staphylococcus aureus and Streptococcus pyogenes were the least isolated from fresh banana fruits with an occurrence of (1) 25% while Escherichia coli was the most isolated with an occurrence of (2) 50% **(Table 6).**.

Staphylococcus aureus and Streptococcus pyogenes were the least isolated from spoilt banana fruits with an occurrence of (1) 25% while Escherichia coli was the most isolated with an occurrence of (2) 50% **(Table 7).**

Table 1 : Bacteria count (cfu/g)[4] of the samples

Sample	Bacteria count (cfu/g)
F1	4.5×10^5

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F2	3.6×10^5
S1	1.49×10^6
S2	1.21×10^6

Table 2: Morphology and Biochemical Characteristics of Bacteria Isolates

Cultural Characteristics	Cell Shape	Gram Reaction	Catalase test	Coagulase test	Indole test	Suspected organisms
Circular pale yellow colonies with smooth shiny surface.	Cocci	+ve	+	+	-	*Staphylococcus spp*
Circular pale yellow colonies with smooth shiny surface.	Cocci	+ve	-	-	-	*Streptococcus pyogenes*
Circular white to grayish white colonies with smooth small surfaces	Rod	-ve	+	-	+	*Escherichia coli*

KEY;

+	=	Positive
-	=	Negative
+ve	=	Gram Positive
–ve	=	Gram Negative

Table 3; Morphological features of fungal isolates

S/N	Macroscopic Features	Microscopic Features	Probable Organism
1.	Pinkish shining smooth in front view and pink colour at the reverse view	Simple branched aseptate hyphae with conida lined at the tips of each hyphae	*Fusarium spp*
2.	Gray green colony	Presence of unbranched conidiophores.	*Aspergillus fumigatus*
3.	Yellow-green cotton colonies.	Aseptate hyphae with conidia lined at the tips of the hyphae	*Aspergillus flavus*

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4.	White cotton candy fluffy appearance	Branched sporangiphores and rhizoids absent	*Mucor spp*
5.	White cotton candy dense growth. Reverse white	Well-developed rhizoids at the point on the stolon and unbranched sporangiphores	*Rhizopus spp*
6.	Appear smooth, creamy and white to off-white colour	Appear as single cells and in large colony	*Candida spp*

Table 4; Percentage Occurence of Fungal Isolates for Fresh Banana

FUNGAL ISOLATES	NO. OF OCCURENCE	% NO. OF OCCURENCE
Fusarium spp	2	25
Aspergillus fumigatus	0	0
Mucor spp	1	12.5
Rhizopus spp	2	25
Candida spp	2	25
Aspergillus flavus	1	12.5
TOTAL;	8	100

Table 5; Percentage Occurrence of Fungal Isolates For Spoilt Banana

FUNGAL ISOLATES	NO. OF OCCURENCE	% NO. OF OCCURRENCE
Fusarium spp	1	10
Aspergillus fumigatus	2	20
Mucor spp	2	20
Rhizopus spp	2	20
Candida spp	2	20
Aspergillus flavus	1	10
TOTAL;	10	100

Table 6; Percentage Occurrence of Bacteria Isolates for Fresh Banana

BACTERIA ISOLATES	NO. OF OCCURENCE	% NO. OF OCCURRENCE
Staphylococcus spp	1	25
Streptococcus pyogenes	1	25
Escherichia coli	2	50
TOTAL;	4	100

Table 7; Percentage Occurrence of Bacteria Isolates for Spoilt Banana

BACTERIA ISOLATES	NO. OF OCCURENCE	% NO. OF OCCURRENCE
Staphylococcus spp	1	25
Streptococcus pyogenes	1	25

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Escherichia coli	2	50
TOTAL;	4	100

DISCUSSION

The findings from this study have shown that both fungi and bacteria can be found in banana fruits. This study shows that the fungi *Fusarium spp, Aspergillus flavus, Aspergillus fumigatus, Rhizopus spp*, *Mucor spp and* yeast isolated as *Candida spp* are important agents of spoilage of banana fruit. Some of the fungi isolated in this study are known to produce several toxic metabolites and they can produce a mycotoxin which is a very important toxin, as they pose hazard to human and animal health, thus extra care should be taken during personnel handling of these fruits such as, harvesting, cleaning, sorting, packaging, transport and storage.

From this Study, it was also seen that the range of bacteria count was from 4.5 x 10^5CFU/ml to 1.21 x 10^6 CFU/ml, indicating higher amounts of bacteria population in the spoilt samples. The bacteria identified in this study are *Staphylococcus aureus, Streptococcus* and *Escherichia coli*. Sufficient moisture, abusive temperature and adequate time ensure a continuing increase in the bacteria population. They are all associated with plant where they are known to cause fruit diseases of the rot, which leads to deterioration of the fruit. *Escherichia coli* are indicator of feacal contaminated products, therefore, sellers of fruits processors may be sources of these microbial contamination. *Staphylococcus aureus* which was isolated from some of the samples is a micro flora of nostrils, skin and hand of man. It might have come from the trader or the air (Argudn, et al, 2010).

CONCLUSION

Banana production and storage is faced with a lot of challenges, especially in prolonging the shelf life. Banana (*Musa species*) yields an environment which is suitable for the growth of organisms, this leads to its microbial spoilage.

This study shows that microorganism such as Bacteria and Fungi causes deterioration and spoilage of banana fruits and consumption of some of the infected bananas fruit causes disease in man as a result of toxics secreted by fungi.

Some of the common contaminating microorganisms which were isolated from the banana fruit already spoilt could come from the farm while some of the microorganisms were acquired as postharvest contaminant probably in temporary storage and in transit which finally manifest their spoilage activities in the market.

The careless handling and storage of banana fruit which leads to bruise or cut on the banana peel causes spoilage of the fruit as a result of penetration and activities of microorganism. Storing the banana in a dirty environment or the use of dirty tables and rags used in covering tables increases the incidence of contamination.

Various storage condition and environmental situation also increases the rate of spoilage of the fruit.

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