

Isolation And Identification of Microorganisms from Some Spoiled Fruits and Vegetables Sold within the Federal Polytechnic of Oil and Gas Bonny Island, Rivers State

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Abstract

This study was conducted to isolate and identify microorganisms from some spoiled fruits and vegetables vended within the environment of Federal Polytechnic of Oil and Gas Bonny Island, Rivers State. Microbial counts of the spoiled fruits and vegetables are 2.30×10^5 cfu/ml (tomato), 8.00×10^5 cfu/ml (apple), 1.70×10^7 cfu/ml (onion) and 1.50×10^9 cfu/ml (banana) for total heterotrophic bacteria count, while the fungal counts were 3.00×10^3 cfu/ml (apple), 4.00×10^3 cfu/ml (onion), 5.00×10^3 cfu/ml (tomato) and 7.00×10^3 cfu/ml (banana). The fruits and vegetables were isolated for bacteria, and *Staphylococcus* spp., *Pseudomonas* spp., *Bacillus* spp., *Micrococcus* spp., *Escherichia coli* and *Proteus* spp. *Bacillus* spp. were observed to be dominant. The fungal organisms observed were *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Saccharomyces* spp., *Rhizopus* spp. and *Mucor* spp.

Keywords: Isolation of microorganism, Tomato, Onions, Apple, Banana

INTRODUCTION

Globally, Fruit are good dietary source of nutrients, micronutrient, vitamins and fiber for human, hence they are very essential for the overall wellbeing of man. The consumption of fruits has increased over the years in many parts of the world (Mahale *et al.*, 2008).

In Nigeria, fruits are popularly displayed completely exposed for sales along busy and major streets and hawked by street food vendors in motor parks and markets. Well balanced diets, rich in fruits and vegetables, are especially valuable for their ability to provide vitamins C and A deficiencies and are also reported to reduce the risk of several diseases (Allamin *et al.*, 2015). Fruits and Vegetables are rich source of vitamins as well as minerals and fibers, thus are essential to sustain a healthy lifestyle. They are also a rich source of antioxidants thus are essential to protect us from various diseases (Kalia and Gupta, 2006). Vegetables are consumed on a daily basis and their consumption is also key area for maintaining a balanced diet. One of the major risks for the preservation of fruits and vegetables is that they have a very short shelf life and they are exposed to the microbial contamination if contacted through soil, dust and water. Thus, preservation of fruits and vegetables are very much important for consumption for a longer duration. Many countries are engaged in the production of fruits and vegetables to a larger extent. India being an agricultural rich country produces fruits and vegetables in a huge scale. India is the second largest producer of fruits and vegetables after China. According to Kumar *et al.* (2011), Food and agricultural organization (FAO) data shows that India has

produced about 76424.2 tons of fruits, 156325.5 thousand tons of vegetables and 388269.2 tons of food grains in 2011. Even due to enormous production of fruits and vegetables, during transportation, handling, storage heavy losses of fruits and vegetables are reported (Zubbair, 2009; Chukwuka *et al.*, 2010; Barth *et al.*, 2013).

The differences in the presence or absence of microorganisms could be due to various factors which can be due to resident microflora in the soil, presence of any non-resident microflora through animal manures (Khatri and Sharma, 2018). Microorganisms spoil them and thus leads to a great loss to the Mankind. Spoilage leads to change in texture, taste and which can't be eatable (Akinmusire, 2011).

MATERIALS AND METHODS

Study Area

The study was carried out within the federal Polytechnic of Oil and Gas and her surroundings. Bonny (or Ibani) is an island town and a Local Government Area in Rivers State in southern Nigeria, on the Bight of Bonny, Bonny is near Port Harcourt; Ferries are the main form of transport to and from the Island. According to 2006 census, Bonny has an estimated population of 214,983 inhabitants. Bonny covers a wide area of about 645.60 km². The Local Government Area lies on latitude 4°26'N and longitude 7°10'E. The local government has two urban centers; Bonny and Finima. The predominant occupations include farming (fishing), trading and civil/public servants.

Sampling and Data Collection

The samples comprised of two fruits (apple and banana) and two vegetables (tomato and onion) which was purchased from three different sites vendors within the Federal Polytechnic of Oil and Gas and her surrounding in Bonny Local Government Area of River State.

All samples were collected in sterile polythene bags and transported to the laboratory, Science Laboratory Technology, Federal Polytechnic of Oil and Gas, Bonny for the isolation and identification of microorganisms which are responsible for their spoilage.

Isolation of fungi

Isolation of fungi from the spoiled fruits and vegetables was done following standard procedures. The samples were washed with sterile distilled water and ultimately surface sterilized with 1% of Calcium Hypochlorite [Ca(OCl)₂] and with the help of sterilized sharp razor, tissues adjacent to the diseased portion were cut and placed into Potato Dextrose Agar (PDA) media which was supplemented with penicillin (100000 units/L) and streptomycin (0.2 g/L). The plates with the sterilized tissues were incubated for a week at 26±2°C until the growth of the fungi was not observed. The fungi growing out from the adjacent tissues were identified following standard identification manuals (Gilman, 1957; Barnett and Hunter, 1988).

Isolation of bacteria

Isolation and identification of bacteria from the spoiled fruits and vegetables collected from three different sites within the Polytechnic environment was done following serial dilution method (Aneja, 2009). All the collected samples of fruits and vegetables were crushed with a blinder which was sterilized earlier and a suspension from them will be prepare by adding distilled water up to 100 ml, which was diluted serially to 10⁻¹ to 10⁻⁵ dilutions. From each of

the dilution about 100 μ L were taken and streak all over on nutrient agar medium (NAM) plates which were already supplemented with amphotericin B (10 μ g/mL) to prevent any contamination from fungi. The plates were then incubated at 37°C for about 24 hours until the growth of bacteria was not observed. The grown colonies were then subculture on NAM slants at 4°C for further use (Chaudhary and Dhaka, 2016).

The bacterial isolates were identified following the Manual of Systematic Bacteriology (Claus and Berkeley, 1986). The morphology of the isolated bacteria was examined following Gram's staining and on the basis of some biochemical test viz., Methyl Red and Catalase test (Cappuccino and Sherman, 2005).

RESULTS AND DISCUSSION

Microbial Counts of Spoiled Tomato, Onion, Apple and Banana

The microbial counts of spoiled tomato, onion, apple and banana are shown in Table 1 below.

The total heterotrophic bacterial (THB) counts ranged from 1.50x10⁹-2.30x10⁵ cfu/ml, with the spoiled tomato sample having the lowest count, and the spoiled banana sample having the highest. There was significant difference (p<0.05) between the samples in their THB counts. Orpin *et al.* (2017) reported total bacterial count of 1.04x10⁵-5.92x10⁵ cfu/ml for onions, and differs from 1.70x10⁷ cfu/ml reported in this study. The tomato sample however recorded 2.30x10⁵ cfu/ml and was similar to the report by Orpin *et al.* (2017). The bacterial counts of the spoiled frits were above the acceptable limit for dried fruits (10⁵) (FAM, 1995).

The fungal count ranged from 7.00x10³-3.00x10³ cfu/g with the spoiled apple sample recording the lowest count, and the spoiled banana sample having the highest counts. There was no significant difference (p<0.05) between samples. The fungal counts were above limit (10²) (FAM, 1995), and was lower than 1.0x10⁵-5.80x10⁵ cfu/ml reported by Orpin *et al.* (2017) for onions sampled from different markets.

Table 1: Microbial Counts of Spoiled Tomato, Onion, Apple and Banana

Counts (cfu/ml)	Tomato	Onion	Apple	Banana
THB	2.30 ^d x10 ⁵	1.70 ^b x10 ⁷	8.00 ^{cd} x10 ⁵	1.50 ^a x10 ⁹
Fungal count	5.00 ^{ab} x10 ³	4.00 ^{bc} x10 ³	3.00 ^c x10 ³	7.00 ^a x10 ³

Values are means \pm Standard Deviation of duplicate determinations. Means in the same rows with different superscript are significantly different at p<0.05

Keys:

THB= Total heterotrophic Bacteria

Phenotypic and Biochemical Characterization of Isolates from Spoiled Tomato, Onion, Apple and Banana

As shown in Table 2, a total of about 6 isolates were isolated from spoiled fruits and vegetable samples and includes *Staphylococcus spp.*, *Pseudomonas spp.*, *Bacillus spp.*, *Micrococcus spp.*, *Escherichia coli* and *Proteus spp.* *Bacillus spp.* showed to be the most dominant in the samples, while *Proteus spp.* was found to be the least dominant and appeared only in onion. *Staphylococcus spp.*, *Pseudomonas spp.* appeared only in tomato and onion. *Pseudomonas spp.* and *Bacillus spp.* were dominant bacterial isolates collected from spoiled fruits and vegetable collected from local and supermarket (Raja *et al.*, 2012). Devi and Gogoi (2021) also reported *Bacillus spp.* to be dominant in spoiled tomato and onions and this finding is in agreement with this study. According to Chaudhary and Dhaka (2016) *Bacillus sp.* was dominant in spoiled fruits and vegetable collected from local market. Occurrence of *Staphylococcus aureus*, a pathogenic organism in most of the spoiled fruits and vegetables is of major concern for public health.

Table 2: Phenotypic and Biochemical Characterization of Isolates from Spoiled Tomato, Onion, Apple and Banana

Isolates	Colony Characteristics		Microscopy	Motility	Catalase	Glucose	Mannitol	Lactose	Sucrose	Oxidase	Methyl red	Voges Proskauer	Indole	Citrate	Tentative Genera	Tomato	Onion	Apple	Banana
A.	Golden yellow	round smooth	+ clustered cocci	-	+	+	+	+	+	-	+	+	+	+	<i>Staphylococcus</i> sp	+	+	-	-
B.	Green	small round moist	-ve rods	+	+	-	+	-	-	+	-	-	-	+	<i>Pseudomonas</i> sp	+	+	-	-
C.	Milky	flat dry	+ chained rods	+	+	+	+	-	+	-	-	+	-	+	<i>Bacillus</i> sp	+	+	+	+
D.	Yellow	small round	+ve small cocci	-	+	-	+	+	+	-	-	-	-	+	<i>Micrococcus</i> sp	+	-	-	+
E.	Circular, smooth,	dark purple colour	-ve rods	+	+	+	-	+	+	-	+	-	+	-	<i>Escherichia coli</i>	-	+	+	+
F.	Pale	small round	-ve rods	+	+	+	-	-	+	-	-	+	-	+	<i>Proteus</i> sp	-	+	-	-

Cultural and Morphological Characteristics of Fungi Isolated from Spoiled Tomato, Onion, Apple and Banana

As shown in Table 3, a total of 6 isolated were isolated from spoiled tomato, onion, apple and banana samples and includes *Fusarium spp.*, *Aspergillus spp.*, *Penicillium spp.*, *Saccharomyces spp.*, *Rhizopus spp.* and *Mucor spp.* *Fusarium spp.* and *Rhizopus spp.* were found to be dominant in the samples, but were not found in apple. *Penicillium spp.* and *Aspergillus spp.* was also dominant but was absent in onion. The least dominant was *Saccharomyces spp.* and was only found in banana. The fungi isolated in this study have been known to cause diseases of human and animals like Ring worm and Aspergillosis which come from the air, wind, water, soil and even the handler (Orpin *et al.*, 2017). They are the sources of highly potent mycotoxins which are hazardous to health. The presence of these fungi in significant numbers in these spoiled fruits and vegetable is therefore a public health risk, and consumption of the fruits and vegetable when spoiled is highly condemned. Dimkpa and Onuegbu (2010) also implicated fungi as contaminants of many agricultural commodities including onions.

Table 3: Cultural and Morphological Characteristics of Fungi Isolated from Spoiled Tomato, Onion, Apple and Banana

Isolate Code	Colony Colour	Type of Soma	Nature of hyphae	Special Vegetative Structure	Asexual spore	Special Reproductive Structure	Probable Organism	Tomato	Onion	Apple	Bannana
A	Whitish yellow colony	Filamentous	Septate.	Mycelium	Chlamydo spores	-	<i>Fusarium sp</i>	+	+	-	+
B	Brownish colony	Filamentous	Septate	Footcell	Globose conidia	Shot conidiospores	<i>Aspergillus sp</i>	+	-	+	+
C	Grey-green colony	Filamentous	Septate	Multicellular mycelium	Conidiospores	Lateral and terminal conidiospores	<i>Penicillium sp</i>	+	-	+	+
D	Moist milk colony	Large single glabose cells	-	Rudimentary pseudomycelieu	Budding cells	-	<i>Saccharomyces sp</i>	-	-	-	+
E	Whitish/ grey fuzzy rapidly growing colony	Filamentous	Aseptate	Sporangiospore	Sporangiospores	Sporangia	<i>Rhizopus sp</i>	+	+	-	+
F	Cotton Blackish colony	Filamentous	Aseptate	Mycelium	Chlamydo spores	-	<i>Mucor sp</i>	+	-	+	-

CONCLUSION

The result of this research show that spoiled apple, tomato, onion and banana are reservoir of both fungal and bacterial microorganisms which have been known to cause spoilage. Some of the organisms isolated, *Staphylococcus spp.*, *Pseudomonas spp.*, *Bacillus spp.*, *Micrococcus spp.*, *Escherichia coli* and *Proteus spp.* *Bacillus spp.* with *Bacillus spp.* being the most dominant bacteria. *Mucor spp.* *Fusarium spp.*, *Rhizopus spp.*, *Penicillium spp.* and *Aspergillus spp.* were the dominant fungi observed in the samples. dominant but was absent in onion. The least dominant was *Saccharomyces spp.* The presence of these fungi in significant numbers in these spoiled fruits and vegetable is therefore a public health risk, and consumption of the fruits and vegetable when spoiled is not advisable.

CONFLICT OF INTEREST

The authors declare that no conflict of interest exists.

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