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Isolation and Identification of Bacteria from Peeled and Ready to Eat Pineapple (*Ananas Comosus*) Fruits Retailed at Eke Awka Market, Anambra State, Nigeria

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ABSTRACT

Pineapple (*Ananas comosus*) is an indispensible fruit that is cherished by many people due to its huge health benefits. It is peeled and sold in many markets and road sides for easy accessibility. The presence of bacteria in the peeled and ready to eat fruits was checked in this study. Peeled, sliced and cellophane packaged pineapple fruits were purchased from Eke Awka Market in Anambra State Nigeria. Nutrient agar was used to carry out bacterial isolation using pour plate technique. Results showed that colony count of the pineapple fruits ranged from 3.5 - 9.5^{-2}cfu/ml of the rinsed water. The isolates were identified on the basis of their colony and morphological features as well as biochemical and sugar fermentation tests. Gene sequencing was used to confirm the species of some of the isolates. A total of six bacteria species were isolated and identified with frequencies as *Streptococcus spp* (13.9%), *Pseudomonas aeruginosa* (22.2%), *Staphylococcus aureus* (25.0%), *Micrococcus luteus* (11.1%), *Escherichia coli* (19.5%) and *Staphylococcus epidermidis* (8.3%). *Staphylococcus aureus* has the highest frequency followed by *Pseudomonas aeruginosa*. *Staphylococcus epidermidis* has the least frequency. Almost all the isolates are pathogenic in nature and their presence in the consumable fruits indicates possible health problems to the consumers. The presence of *E. coli* indicates direct or indirect fecal contamination. Proper handling of pineapple fruits, hygiene and proper storage will help reduce the risk of contamination by these organisms.

KEYWORDS: *Spoilage, Pineapple, fruits, Post harvest loss, Proper storage*

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INTRODUCTION

Fruits are rich source of vitamins and minerals required by the human body for nourishment, growth and metabolism. They are agricultural products which are readily obtained from many markets across the country. Most are eaten raw with little or no processing. Pineapple (*Ananas comosus*) is one of such fruits. It is a vegetative propagated fruit crop that is grown and consumed by almost every country of the world. It is one of the few crops in which cultivars are derived from spontaneous mutations and natural evolution without controlled breeding (Osei-kofi *et al,* 1997). Pineapple is a cylindrical false fruit (pseudo-fruit) of the family Bromeliaceae and consists of a thickened, fleshly, very juicy axis core and inedible, scaly, warty skin, resembling a pine core. Only the polygonal, flattened outsides of the individual fruits are visible at the surface of the multiple fruit (syncarp) (Chaurasiya *et al.*, 2015). The fruit is topped by a crown of prickly leaves. The axis core (central cylinder) in the middle of the false fruit is woody and therefore inedible. They can be used in the preparation of jellies, juice, jams and fruit salad. Microorganisms are naturally present on all food stuff and can also be brought in by outside elements (wind, soil, water, insects, animals, human handling). They can become contaminated during growing, harvesting and transport of the raw materials and/or processing into edible products (Kochhar, 2006).

Pineapples contain high level of sugars and other nutrients and are low pH values which make them particularly prone to bacteria infestation. Bacteria can

survive and grow on pineapples as source of nutrients such as carbohydrates, proteins and fats. Ripe fruits including pineapple are soft and susceptible to infestation by a variety of disease-causing organisms including bacteria. Softening due to ripening is the major cause of post harvest losses incurred on most fruits. Pineapple infection may occur during the growing season, harvesting, handling, transport, post harvest processing and marketing conditions or after purchase by the consumer. Another major source of contamination is the washing water (Khali and Mazher, 1994) used by market retailers who peel the fruits and sell to final consumers.

In developing countries as Nigeria, post-harvest losses are often more severe due to inadequate processing, storage and transportation facilities (Droby, 2006; Khali and Mazher, 1994). Pineapple fruits are delicious and contain nutrients such as water, carbohydrates, sugars, beta-carotene, gallic acid, syringic acid, vanillin, ferulic acid, sinapic acid, coumaric acid, chlorogenic acid, epicatechin, and arbutin (Nwachukwu *et al*.,2008) It contains low amounts of protein and fat, with high quantity of ash, fiber and antioxidants such as flavonoids with vitamin A and C. These antioxidants reduce the oxidative damages that are caused by free radicals and chelating metals. It also contains the enzyme bromelain. This enzyme harbor peroxidase, acid phosphate, several protease inhibitors and organically bound calcium (Nwachukwu *et al*., 2008). The peel is rich in cellulose, hemicelluloses and other carbohydrates. Ensilaging of pineapple peels produces methane which can be used as a biogas. The primary cell wall of pineapples is composed of approximately 10% proteins and 90% polysaccharides which can be divided into three groups: cellulose, hemicelluloses and pectin (Nathalie, 2006). Those with allergies may incorporate pineapple into their diets more regularly to reduce sinus mucus long term (Stah *et al*., 2012).

The consumption of pineapples has been on the increase in due to their health benefits. They are easily accessible, nutritious and relatively cheap (Nwachukwu *et al*, 2008). The increase in consumption has been linked with a parallel increase in food-borne illnesses (Mensah *et al,* 2002). Pineapple fruits are peeled and sold by unlicensed street vendors with poor education and lack of training in food hygiene (Barro *et al,* 2006). These fruits are usually displayed on benches and in baskets for prospective customers in the open markets until sold, thereby exposing them to further bacterial infections. There is therefore need to assess the microorganisms that invade them and seek ways of improving their method of display in the market with little or no contamination because consumption of spoilt pineapple fruits have been linked to several gastrointestinal problems and some forms of food borne diseases.

Pineapple fruit on the plant (Source: https://en.m.wikipedia.org/wiki/Pineapple)

MATERIALS AND METHODS

Fifteen (15) samples of peeled, sliced and displayed pineapple fruits were randomly purchased from different retailers in Eke Awka market, Anambra State using sterile bags and transported to the Department of Applied Microbiology and Brewing laboratory. Nutrient agar and other reagents used were obtained from the laboratory and were of analytical grade.

SAMPLE PREPARATION:

The fruits were labeled according to the place of purchase assigning the codes SP1 to SP15. Using a sterile knife, 1g of each sample was cut and aseptically crushed in a mortar and transferred in a test tube containing 20ml distilled water. The tube was shaken vigorously for five seconds and from the liquid, 1ml was aseptically transferred into 9ml of sterile distilled water in another test tube to give 10^{-1} dilution using a sterile pipette. The serial dilution was made into the second test tube to get a dilution of 10^{-2} which was subsequently cultured. Nutrient agar was prepared according to the manufacturer's specification, autoclaved and allowed to cool to 45°C.

ISOLATION OF ORGANISMS

Pour plate method as described by Modi *et al*., (2009) to determine the bacterial count was done. Using a sterile pipette, 0.1ml of each dilution was dispensed in a sterile petri dish and the cooled nutrient agar was poured, swirled to mix with the drop of the sample suspension and allowed to set on a bench. The plates were incubated at 37^{0}C for 24 hours after which total bacterial colonies were counted. Plates that contain 30-300 colonies were counted and colony forming unit (cfu) was calculated using the number of colonies multiplied by the dilution factors.

BACTERIA IDENTIFICATION

Colonies from nutrient agar plates were sub-cultured on freshly prepared nutrient agar plates by streaking and incubated at 37^0C for 24 hours to obtain pure cultures. The pure colonies were stored on slant in bijou bottles and stored in the fridge at -4°C (Arshad *et al.*, 2016). The following tests for the identification were conducted as prescribed by Cheesbrough, (2003):

Gram staining

A drop of distilled water was placed on a clean grease free glass slide and a colony in isolates was picked with a sterilized wire loop and emulsified. The glass slide was passed over the flame three times to heat fix. The smear was flooded with crystal violet for 60seconds and rinsed with distilled water. Lugol's iodine was added, then decolourized with acetone and rinsed immediately with distilled water. The smear was counter stained with Safarin for 1-2minutes and rinsed with distilled water. The smear was then allowed to air dry after which oil immersion was added and viewed under microscope using X100 objective lens.

Coagulase test

A drop of physiological saline was put on a clean glass slide, a loopful of 24 hr culture of the isolate was mixed with the drop of saline. Then a drop of human plasma was added into it and smeared to make a suspension. The slide was kept for 1 minute and the presence of agglutination was checked.

Catalase test:

A test tube containing Hydrogen peroxide solution was shaken to expel the dissolved oxygen. One drop of the solution was dropped on a clean glass slide followed by the addition of loopful 24hours old inoculums. The drop of hydrogen peroxide and the inoculums were smeared on the slide and examined.

Citrate test

This was done as described by Cheesbrough, (2003). This detects the ability of an organism to use citrate as the sole source of carbon. Simon citrate agar was prepared by weighing 2.5g of Sodium citrate, 1.5g of Ammonium phosphate, 0.2g of Magnesium sulphate, 1g of Potassium dehydrogenate phosphate and 0.1g of Bromothymol blue and dissolved in 1litre of distilled water, homogenized and dispensed in test tubes then corked with cotton wool. A speck of each isolate was inoculated into medium and incubated at 37°C for 72hours.

Indole Test

This test was done as described by Cheesbrough, (2003). Peptone water of 1.5g weight was dispensed into 250ml capacity conical flask. 100ml of distilled water was gradually added and shaken. It was then enriched with 1g of Tryptophan and heated on hot plate to homogenize and finally dispensed in to test tubes and corked for sterilization in the autoclave at 121°C for 15minutes. A speck of each isolate was inoculated into 5ml of sterile peptone water enriched with 1% Tryptophan in test and was incubated at 37°C for 48hours to the culture, 0.5ml Kovac's indole reagent was added and gently shaken.

Methyl Red Test (MR Test):

This was carried out as described by Modi *et al.,* (2009) as described by Christopher, (2015). Phosphate buffered glucose peptone medium was prepared by weighing 0.5g of peptone, 0.5g of glucose and 0.5g of dipotassium hydrogen phosphate (K_2HP0_4) into 10ml of distilled water and heated on the hot plate to completely dissolve and dispensed into test tubes, corked with cotton wool and aluminium foil and sterilized at 121^0C for 15minutes. A speck of each isolate was inoculated at 37^0C for 48hours. Few drops of methyl red were added to the culture.

Motility

This was done as described by (Kochhar, 2006). A speck of each isolate was stabbed into Triple Sugar iron agar and incubated at 37°C for 24 hours. Motility was observed by spread of the organism outwards form the stab area. The media constitution was adjusted to suit that of motility test by adding more than the required quantity of water.

Test for sugar utilization

To each 10ml of peptone water, 1.5g of each sugar was separately dissolved into it and few drops of bromocresol green was added into it, then it was dispensed into various test tubes containing inverted durham tubes for gas collection and labeled. The tubes were plugged with non absorbent cotton wool and sealed with aluminum foil before being sterilized. After sterilization, the tubes were allowed to cool and den aseptically inoculated with the isolates culture using a sterile wire loop. The tubes were incubated for 48hours at 30^0c. Acid production was indicated by change in color and gas production was indicated by the presence of air bubbles at the sealed end of the inverted durham tubes (Chaurasiya *et al*., 2015).

RESULT

The results from the study are shown below. Table 1 presents the total viable bacteria count from the different pineapple samples. The colony count ranged from 3.5 to 9.5^{-2}cfu/ml of the shaken liquid. The count is high and may surpass the acceptable limit of bacteria in consumable fruits. Table 2 shows the frequency and percentage occurrence of the bacteria isolates with *Staphylococcus aureus* having the

highest percentage frequency of 25.0%. The bacterial isolates CA to CF were identified as *Streptococcus spp* (CA), *Pseudomonas aeruginosa* (CB), *Staphylococcus aureus* (CC), *Micrococcus luteus* (CD), *Escherichia coli* (CE), *Staphylococcus epidermidis* (CF) as shown in Tables 3 and 4.

Table 1: Total viable count of bacteria isolates from the different pineapple samples

S/N	Pineapple Samples	cfu/g (10^{-5})
1	Sp1	7.5
2	Sp2	5.0
3	Sp3	9.2
4	Sp4	4.6
5	Sp5	8.0
6	Sp6	7.2
7	Sp7	6.5
8	Sp8	3.5
9	Sp9	6.0
10	Sp10	8.5
11	Sp11	7.0
12	Sp12	9.5
13	Sp13	5.5
14	Sp14	4.8
15	Sp15	3.5

Sp = Different pineapple samples

Table 2: Frequency and Percentage frequency of occurrence of the isolates from the pineapple samples

S/N	Isolated Organism	Frequency	Percentage Occurrence of Isolates (%)
1	*Streptococcus spp*	5	13.9
2	*Pseudomonas aeruginosa*	8	22.2
3	*Staphylococcus aureus*	9	25.0
4	*Micrococcus luteus*	4	11.1
5	*Escherichia coli*	7	19.5
6	*Staphylococcus epidermidis*	3	8.3

Table 3: Cultural and Morphological Characteristics of bacteria isolated from the retailed pineapple samples

S/N	Isolated bacteria	Macroscopic colony morphology	Gram staining reaction	Shape and arrangement	Motility
1	*Streptococcus spp* (CA)	Tiny white, opaque Circular and convex	+VE	Cocci in clusters	-
2	*Pseudomonas aeruginosa* (CB)	White, Opaque	-VE	Rods in singles and clusters	-
3	*Staphylococcus aureus* (CC)	Golden yellow, large, circular, convex, undulated	+VE	Cocci in clusters	-
4	*Micrococcus luteus* (CD)	Yellow, circular, smooth and soft	+VE	Cocci in irregular clusters	-
5	*Escherichia coli* (CE)	White, large smooth, opaque, lobate, entire margin	-VE	Rods in scattered arrangement	+
6	*Staphylococcus epidermidis* (CF)	White, shiny and irregular shaped	+VE	Cocci in clusters	-

Key: + = Present, - = Absent, +VE = Positive, -VE = Negative
CA to CF = different bacteria isolates

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Table 4: Biochemical Characteristics of identified bacteria isolates

S/N	Biochemical test\	Bacteria Isolates					
		CA	CB	CC	CD	CE	CF
1	Carbohydrate Fermentation						
	➢ Glucose	A	A	A	-	A	AG
	➢ Sucrose	A	-	A	-	A	AG
	➢ Lactose	A	-	A	-	AG	A
	➢ Maltose	A	-	A	-	AG	AG
	➢ Mannitol	A	A	AG	-	AG	-
	➢ Dextrose	A	A	AG	-	A	AG
	➢ Fructose	A	A	AG	-	AG	AG
	➢ Sorbitol	A	-	-	-	AG	AG
	➢ Galactose	A	-	-	-	AG	-
2	Iodine test	+	-	-	-	+	-
3	Citrate test	-	+	+	+	-	-
4	Catalase test	+	+	+	_	+	+
5	Coagulase test	-	-	+	-	-	-
6	Methyl red test	-	-	+	-	-	+
7	Indole test	-	+	-	-	-	+
	Identified bacteria isolates	*Streptococcus spp*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Micrococcus luteus*	*Escherichia coli*	*Staphylococcus epidermidis*

AG = Acid and Gas production, **A** = Acid production, **+** = Present, **-** = Absent

DISCUSSION

Fruits like pineapples are very important because of their high dietary and nutritional qualities. The prevalence of some bacteria demands that appropriate control measures should be employed if farmers expect a beneficial outcome of their product. Adequate microbiological knowledge and handling practices of these products would therefore help to minimize wastes due to deterioration of fruits (Modi *et al*., 2009). Proper measures should be adopted while handling fruits to limit the level of microbial contamination. It is therefore important that the farmers and stakeholders properly transport the fruits into bags, the marketers and consumers take necessary precaution to prevent contamination and also try to create an environment that will not encourage the growth and multiplication of microorganisms.

A total of 15 pineapple samples purchased from different retailers all contain one form of bacteria or the other. Six different bacteria species were isolated from them. All the bacteria isolated in this study have previously been isolated from pineapple in other studies both in Nigeria and elsewhere (McKenzie and Gene, 2010, Omemu and Bankole, 2005, Tambekar and Mundhada, 2006, Uzeh *et al*., 2009). This shows that sweet fruits such as pineapple can harbor some pathogenic bacteria. Some of the isolates like *Staphylococcus aureus* are implicated in food poisoning while *E. coli* can be among the members that that casse gastroenteritis when ingested in a high load. The percentage frequencies of bacteria in this study are similar to those obtained in other studies in (Uzeh *et al*., 2009; Bukar *et al*., 2010). These microorganisms present in the pineapple samples may be a direct reflection of sanitary quality of the soil, harvesting, transportation, storage and processing of the product (Bhunia, 2007). The bacterial isolates identified in this study; Streptococcus spp (CA), Pseudomonas aeruginosa (CB), Staphylococcus aureus (CC), Micrococcus luteus (CD), Escherichia coli (CE) and Staphylococcus epidermidis (CF) are consistent with the findings of previous studies (Nwachukwu and Chukwu, 2013) who isolated same species from pineapple juice. Presence of E. coli indicates recent contamination by fecal matter and possible presence of other enteric pathogens known to be causative agents of food borne gastroenteritis and bacterial diarrhea (Jiwa, *et al.,* 2011). A number of studies from different countries have shown the presence of E. coli, coliforms and a variety of microorganisms like streptococcus pyogenes, staphylococcus spp, micrococcus spp e.t.c, (Nichols *et al*.,2000), (Lateef *et al*., 2006) (Amusa and Ashaye, 2009). This study has supported the work by Duane, (2006) who found out that microorganisms causing diseases are present in the ripe fruits and this is of public health importance. There is need therefore to create awareness to these retailers on good hygiene

especially displaying their fruits in show glasses rather than displaying on an open table.

CONCLUSION

Ripe fruits can be a good source of nutrient to both humans and microbes. There is great need to retail fruits like pineapple in sealed containers devoid of air to reduce the rate of contamination by these bacteria.

RECOMMENDATIONS: It is recommended that proper washing of these fruits is essential before consumption. Regulation monitoring of the quality of fruits for human consumption should be introduced to avoid any bacterial pathogen outbreak. Handling and storage quality should also be improved.

REFERENCES

[1] Arshad ZI, Amid A, Yusof F, Jaswir I, Ahmad K and Loke SP (2016). "Bromelain: an overview of industrial application and purification strategies". *Applied Microbiology Biotechnology*. 98 (17): 7283–97.

[2] Barro, N; Iboudo, I. and Faore, A.S. (2006). Hygiene Status, assessment of dishwater, utensils, hands and pieces of money in street food vending sites in Ougadougou, Burkina Faso, *African Journal of Biotechnology*. 5: 1107-1112.

[3] Chaurasiya RS, Sakhare PZ, Bhaskar N, Hebbar HU (2015). "Efficacy of reverse micellar extracted fruit bromelain in meat tenderization". *Journal of Food Science and Technology*. 52 (6): 3870–80.

[4] Christopher Cumo (2015). Foods that Changed History: How Foods Shaped Civilization from the Ancient World to the Present, p. 294.

[5] Droby, S. (2006). Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. *Acta Horticulturae*. 709: 45-51

[6] Duane P. B. (2009). ‘MD-2’ Pineapple Transforms the World's Pineapple Fresh Fruit Export Industry" (PDF). *Pineapple News*. 16: 2–5. Retrieved 26 July, 2018

[7] Khali, L.G.B. and Mazher, K.B. (1994). Files and water reservoirs for bacteria enteropathogens in urban and rural areas in and around labore, Pakistan. *Epidemiology and Infection*. 113: 435-444.

[8] Kochhar, SL (2006). Economic Botany in the Tropics. Macmillan India. p. 203.

[9] McKenzie, Gene (2010). "A Little Bit of History". *Journal of the Bromeliad Society*. 60 (4): 187–189.

[10] Mensah, P; Owusu, K; Yeborah, D. and Abiordey, A. (2002). Street Food in Accra, Ghana, how safe are they? *World Health Organization Bulletin*. 80: 546-554.

[11] Modi, Gunjan M; Doherty, Christy B; Katta, Rajani; Orengo, Ida F (2009). "Irritant Contact Dermatitis from Plants". *Dermatitis.*20 (2): 63–78.

[12] Nathalie, J. (2006). Plant protein inhibitors of cell wall degrading enzymes. *Trends in Plant Science*. 2: 350-367.

[13] Nwachukwu, E: Ezeama, C.F. and Ezeanya, B.N. (2008). Microbiology of polyethylene packaged sliced pineapples sold by street vendors in Nigeria. *African Journal of Microbiology Research.*2: 192-195.

[14] Ogawa, E. M; Costa, H. B; Ventura, J. A; Caetano, L. C; Pinto, F. E; Oliveira, B. G; Barroso, M. E. S; Scherer, R; Endringer, D. C; Romão, W (2018). "Chemical profile of pineapple". *Journal of the Science of Food and Agriculture*. 98 (3): 1105–1116.

[15] Osei-Kofi, F; Amoatey, H.M and Lokko, Y. (1997). Improvement of Pineapple (Ananas comosus) using Biotechnology and mutation breeding techniques. In: Report of 2 FAO/IAEA Research coordination meeting on “invitro techniques for selection of randiation-induced mutant adapted to adverse environmental conditions.” Pp 23-27.

[16] Stahl, J. M; Nepi, M; Galetto, L; Guimarães, E; Machado, S. R (2012). "Functional aspects of floral nectar secretion of Ananas ananassoides, an ornithophilous bromeliad from the Brazilian savanna". *Annals of Botany*. 109 (7): 1243–125