Incidence of Bacteria Associated with Post Harvest Rot of *Colocasia esculenta* LIN (Cocoyam) Tubers Sold in Different Markets in Bukuru, Jos South Local Government Area, Nigeria

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Abstract: The bacteria species associated with post-harvest rot of *Colocasia esculenta* Lin (Cocoyam) tubers sold in Bukuru market of Plateau State were investigated. Fifty (50) Samples of *C. esculenta* were obtained randomly from 3 different Markets within Bukuru and brought for analysis. The tubers were surface disinfect with Sodium Hypo chloride (NaOCl) for 5 minutes. A portion of the rotten sample and the surface tissue were sliced into thin sections and inoculated on Nutrient agar and incubated for 24 hours at 37 °C followed by Subculture for another 24 hours to obtain pure cultures. Pure cultures were examined using microscopic examination and biochemical test to identify the bacteria species while a pathogenicity test was carried out using healthy *C. esculenta* corms and cormels. The study identifies nine different bacteria species associated with post-harvest rot of cocoyam. They include with *Pseudomonas aeruginosa* (16.7 %) *Corynebacterium* spp (4.2 %) *Enterobacter cloacae* (4.2 %), *Staphylococcus aureus* (8.3%) *Escherichia coli* (20.8%), *Bacillus subtilis* (12.5 %), *Shigella* spp (4.2 %) *Salmonella* spp (12.5 %) and *Bacillus pumillus* (12.5 %). The percentage frequency of the identified bacteria species shows that *Escherichia coli* had the highest frequency of occurrence to be 20.8 % while *Corynebacterium* spp, *Shigella* spp *Enterobacter cloacae* had the least percentage of occurrence to be 4.2 % each. The pathogenicity test confirmed that the organisms isolated were the actual causal agents of rots in cocoyam sold in Bukuru market. Efforts should be made to put preventive and control measures, which includes proper handling of tubers during harvest, Post harvest, transportation and storage to reduce loss of the crop.
Key words: Bacteria, Post-harvest, Rot, Cocoyam,

INTRODUCTION

Colocacia esculenta Lin (Cocoyam) is a monocotyledonous herbaceous perennial plant from the family Araceae. It is commonly called Guaza or Walli in Hausa, Lamba in Yoruba, Ede or Akasi in Igbo (Lawson and Kalu, 2007).

Colocasia esculenta and other members of the genus are cultivated as ornamental plants, or for their edible corms, a traditional starchy stable food in many tropical areas. The plant consists of a central corm (lying just below the soil surface) from which leaves grows upward, roots grows downwards while cormels or daughter corms and runners (Stolons) grows laterally, the root system is fibrous and lies mainly in the top one meter of the soil (Onwueme, 1978). *Colocasia esculenta* is known as a food crop which provide high yield of edible roots (Corms) and foliage, it is a tropical food crop that can be grown under flooded or upland region (Chatty *et al*., 2007). According to Brown and Valerie, (2004) Dharma and Ugwuja, (2008) Singh *et al.,* (2012) with the leaves, Corm's and petiole are used as a vegetable. The taro plant is a rich source of carbohydrate, Protein, Minerals and vitamins and has medicinal properties to reduce tuberculosis, ulcers, pulmonary congestion. Beside the medicinal properties, taro corms are utilized in various industries for the preparation of high fructose syrup and alcohols (Misra *et al.,* 2008). However, based on various studies of ethno- botanical evidence suggest that *Colocasia esculenta* originated from south central Asia, probably in India of the Malay peninsula (kolchaar *et al.*, 2006). Wild forms occur in various part of eastern Asia (Purse glove, 1972). Hence South east Asia is said to be an important region for ethnobotanical and genetic diversity of *Colocasia esculenta*. From its Centre of origin, it spread East ward, to the rest of Asia and to China, Japan and the Pacific Island from Asia it spread west ward to Arabia and the Mediterranean region it arrived on the east coast of Africa 2,000 years ago.

Today *Colocasia esculenta* is said to be pan tropical in its distribution and cultivation. The bulk of its production is in Africa (Spore, 2003). West Africa which is the highest area of cultivation accounts for the greatest quantity of its production. According to FAO (2006), Nigeria is the largest producers of cocoyam in the world, accounting for about 37% of the world output estimated to be annual production of 5.49 million metric tons, followed by Ghana which produces 31% conversely.

Eze and Okorji (2003) documented that Nigeria account for 40% of the total world output of Cocoyam, thus from the evidence, the cultivation of cocoyam in Nigeria is declining. This research seeks to identify bacteria species causing post-harvest rot of *Colocasia esculenta* sold in Bukuru Market in Jos.

MATERIAL AND METHODS

Sources of Cocoyam

Fifty (50) samples of *Colocasia esculenta* (Cocoyam) tubers (6-7 cm) having signs of rots were obtained from central market Kugiya market and Gyel market within Bukuru metropolis. The samples were collected randomly from three (3) different cocoyam storage stores in sterile paper bags, in the morning between 8-10 am and brought to the Botany Laboratory II for analysis. Freshly harvested cocoyam corms were also obtained in paper bag from the same storage stores for the pathogenicity test.

Isolation of bacteria pathogens from Cocoyam Cormels.

Cocoyam tubers and cormels were surface sterilized (to remove surface contaminants) in 5 % sodium hypochloride and rinsed in sterile distilled water for five minutes. Sterilized blade (forceps) was used to cut Cocoyam into sections of approximately 2mm cubes from the tissue of the junction between healthy and infected portion. They were inoculated on solid nutrient agar in sterile petri dishes and the plates were incubated at 37°C for 24 hours, then examined for the growth of bacteria. The mixed cultures were sub-culture on fresh solid nutrient agar on petri dishes to obtain pure cultures

Biochemical Test for the Identification of the Bacteria species.

The pure cultures were subjected to biochemical tests for the identification of the bacteria species using:

Motility test: Touch or stab with a needle straight in and straight out of the center of the tube half way down to a colony of young (18-24 hours) culture growing on agar medium. Incubate at 35- 37 ℃ and examine daily for up to 7 days. Observe for a diffuse zone of growth flaring out from the line of inoculation. Positive: Diffuse, hazy growths that spread throughout the medium rendering it slightly opaque. Negative: Growth that is confined to the stab-line with sharply defined margins and leaving the surrounding medium clearly transparent.

Catalase Test: Transfer or pour 1-2ml of hydrogen peroxide solution into a test tube. Using a sterile wooden stick or a glass rod, take several colonies of the 18 to 24 hours test organism and immerse in the hydrogen peroxide solution. Observe for immediate bubbling. Positive: Copious bubbles produced, active bubbling. Negative: No or very few bubbles produced.

Oxidase test: A strip filter paper is soaked with a little freshly made 1 % solution of the reagent (tetramethyl-p-phenylene-diamine dihydrochloride). A speck of culture is rubbed on it with a platinum loop. A positive reaction is indicated by an intense deep-purple blue, appearing within 5-10 seconds, a “delayed positive” reaction by colouration in 10-60 seconds, and a negative reaction by absence of colouration or by colouration later than 60 seconds.

Indole Test: Take a sterilized test tubes containing 4 ml of tryptophan broth. Inoculate the tube aseptically by taking the growth from 18 to 24 hours culture. Incubate the tube at 37 ℃ for 24-28 hours. Add 0.5 ml of Kovac’s reagent to the broth culture.

Observe for the presence or absence of ring. Positive: Formation of a pink to red colour (cherry-red ring) in reagent layer on top of the medium within seconds of adding the reagent. Negative: No colour change even after the addition of appropriate reagent.

Citrate Utilization test: Streak the slant back and forth with a light inoculum picked from the center of a well -isolated colony. Inoculate aerobically at 35-37 ℃ for up to 4-7 days. Observe a colour change from green to blue along the slant. Positive reaction: Growth with colour change from green to intense blue along the slant. Negative reaction: No growth and no colour change; slant remain green.

Urease reaction: Streak the surface of urea agar slant with a portion of a well-isolated colony or inoculate slant with 1-2 drops from an overnight brain-heart infusion broth culture leave the cap on loosely and incubate the tube at 35 -37 ℃ in ambient air for 48 hours to 7 days. Examine for the development of a pink colour for as long as seven days. Positive reaction: Development of an intense magenta to bright pink colour in 15 minutes to 24 hours. Negative reaction: No colour change.

Hydrogen Sulfide test: In sulphite indole motility medium: Inoculate the organism into labeled tube by means of stab inoculation. Incubate the inoculated tubes at 37 ℃ for 24-48 hours. Observe for the formation of black precipitate on the medium. Positive result: Blackening on the medium. Negative result: No blackening on the medium.

Gelatin Utilization: Apply nutrient gelatin plate method by stab-inoculate a heavy inoculum of an 18-24 hours old test bacteria onto culture plates prefilled with nutrient gelatin (23g/liter nutrient agar, 8g/liter nutrient gelatin). Incubate inoculated nutrient gelatin plates at 35 ℃ for 24 hours. In some cases, plates are flooded with saturated ammonium sulfate to precipitate unhydrolyzed gelatin, making the clear zones easier to see. Results are often observed within 5-10 minutes after flooding with saturated ammonium sulfate. Positive results show partial or total liquefaction of the inoculated tube (the control tube must be completely solidified) at 4 ℃ within 14 days. On plates, gelatin hydrolysis is indicated by clear zones around gelatinase-positive colonies. Complete solidification of the tube at 4 ℃. On plates, no clear zones around colonies are observed.

Carbohydrate Utilization: Add carbohydrate solution to the broth base and mix it. The preferred carbohydrate concentration is 1%. Aseptically inoculate each test tube with the test microorganism using an inoculating needle or loop. Alternatively, inoculate each test tube with 1-2 drops of an 18–24 hours brain-heart infusion broth culture of the desired organism. Incubates tubes at 35-37 ℃ for 18-24 hours. Longer incubation period may be required to confirm a negative result. Colour change to yellow/yellowish green indicate positive result. The tube containing medium will remain red. Indicating negative result. (Tokuyasu *et al.,* 2012).

Identification of bacteria Pathogens isolated from Cocoyam Cormels

The Gram staining procedure modified by Rueckert and Morgan (2007) was used in the microscopic identification of the bacterial species. The reagents used were crystal violet, Safranine (biological stain) iodine and absolute ethanol, a clean microscope slide was labeled with the code for the unknown organism using a marking pencil. One loopful of unknown bacteria culture was applied on the slide using an inoculating loop. The smear was then heat fixed on the slide by passing it over a flame of the Bunsen burner (Lima-Bittencourt *et al.*, 2007).

The slide with the bacterial smear was held at the edge using a sterile cloth peg over a staining basin. It was then flooded with crystal violet, the primary stain for 40 seconds. The stain was washed off with sterile tap water before applying Gram's iodine for another 20 seconds. The Gram's iodine was washed off with tap water before adding 95% (v/v) ethyl alcohol, drop by drop until the alcohol was clear.

The smear was counter stained with safranin for 40 seconds before it was gently washed off with sterile tap water. The smear was then blotted dry before it was viewed under a microscope on the oil immersion objective lens. The shape of the bacteria and the staining effects were recorded (Gbolagade, 2004).

Pathogenicity test

Medium-sized (6-7 cm) sound cormels of Cocoyam free from wounds were used for testing the pathogenicity of isolates. The method employed was similar to that described previously for inoculating cocoyam (Maduewesi and Onyike, 1980) and yam (Cornelius, 1998). Cormels were thoroughly washed in running tap water for ten minutes, surface sterilized in 5% sodium hypochlorite solution (commercial bleach) and air-dried for four minutes. Before inoculating, the point of inoculation was wiped with 95% ethanol. Pathogenicity test was carried out using four cocoyam which were surface sterilized with 10% Sodium hypochlorite. A sterile 5mm diameter cork borer was used to make holes on the cormels to a depth of about 8 mm.

The parts of the Cocoyam which was bored out at each point were kept in sterile petri dishes. An agar block from the growing culture of each test isolate (Pure culture) was inoculated into the holes, after the inoculation the parts of the Cormels bore out were carefully replaced and sealed with sterile Vaseline to prevent contamination and labelled accordingly. A control experiment which bore no isolate was also set-up (inoculated with 1ml of sterile distilled water. All the Cormels were incubated for 10 days in a humidity chamber under room temperature. The Cormels were examined daily for evidence of rot such as Softening, discoloration and offensive odour. At the end of the 10 days incubation period, the Cormels were carefully cut open along the line of inoculation to expose the region of the Cormels which were then examined for rot. Where positive the length and girth of the rot area and those of the entire Cormels were measured and recorded.

RESULTS

Bacteria Isolated from Rotten Tissues and Surface of Cocoyam

A total of nine (9) bacteria isolate were isolated and identified from both the surface of cocoyam cormels and the internal rotten tissue. The bacterial isolates were *Bacillus subtilis, Staphylococcus aureus, P. aeruginosa, E. coli, Corynebacterium* sp*, Salmonella* sp*, Shigella* sp, *Bacillus pumilus. E. coli* were identified twice both on the internal rotten tissue and the surface of the cormels. Generally, the internal tissues had more bacteria diversity than the surface of cocoyam.

Frequency of occurrence of Bacteria in the Sample Markets showed that the Central Market had the highest frequency of bacteria occurrence (50 %). While Kugiya Market and Gyel Market had similar percentage occurrence of the bacteria (25.0 %) each. *E. coli* was the most frequently isolated bacteria while *Shigella* spp and *Corynbacterium* spp were the least frequent bacteria isolated. (Table 2).

The percentage frequency of the identified bacteria species showed that *Escherichia coli* had the highest percentage frequency of occurrence with 20.8 % while *Corynebacterium* spp, *Shigella* spp, *Enterobacter cloacae* had the least percentage of occurrence of 4.2 % each (Table 3)

Pathogenicity test

Pathogenicity test revealed that *E. cloacae* was the most pathogenic bacteria with percentage area of infection of 14.3 % while *B. pumilus* was least pathogenic with 7.1 % as percentage area of infection. (Table 4)

Table 1 Bacteria Species Isolated from Surfaces and Internal Rotten Tissue of Cocoyam Tubers and Cormel

Bacteria from Surfaces	Bacteria from Internal Rotten tissue
Escherichia coli	*Bacillus subtilis*
Shigella species	*Staphylococcus aureus*
Bacillus pumilus	*Escherichia coli*
Salmonella species	*Pseudomonas aeruginosa*
	Corynebacterium spp
	Enterobacter cloacae

Table 2: Occurrence of Bacteria Species Isolated in relation to different Areas in Bukuru Market

Bacteria Species	Central Market	Kugiya Market	Gyel Market
Pseudomonas aeruginosa	1	1	2
Escherichia coli	3	1	1
Corynebacterium spp	1	0	0
Staphylococcus aureus	0	2	0
Bacillus subtilis	2	0	1
Enterobacter cloacae	1	0	1
Bacillus pumilus	2	0	1
Shigella species	1	0	0
Salmonella species	1	2	0
Total (%)	**50.0%**	**25.0%**	**25.0%**

Table 3 Overall Occurrence of Bacteria Isolates from the sample collection Areas.

Bacteria Species	Number of Isolated Bacteria	Frequency of Occurrence %
Psedomonas aruginosa	4	16.7 %
Esherichia coli	5	20.8 %
Coryn bacterium species	1	4.2 %
Staphylococus aureus	2	8.3 %
Bacillus subtilis	3	12.5 %
Enterobacter cloacae	2	4.2 %
Bacillus pumilus	3	12.5 %
Shigella species	1	4.2 %
Salmonella species	3	12.5%
Total	24	100 %

Table 4 Pathogenicity of the isolated bacteria on healthy cocoyam

Bacteria Species	Discoloration on healthy Cormels	Average area of infection	Percentage area of infection by the bacteria isolates
Pseudomonas aruginosa	Browish-black	3.5	12.5 %
Enterobacter clocae	Brown	4.0	14.2 %
Staphylococcus aureus	Gray to brownish gray	2.5	8.9 %
Corynebacterium sp	Brown	2.5	8.9 %
Bacillys Subtilis	Brown	3.5	12.5 %
Shigella sp	Brown	3.0	10.7 %
Salmonela sp	Gray	3.5	12.5 %
Bacillus pumilus	Brownish-black	2.0	7.1 %
E. coli	Brown	3.5	12.5 %
Total		35	100 %

DISCUSSION

The study showed a variety of bacteria species associated with *C. esculenta* rot in Bukuru Market and it environ. The bacteria species includes *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Corynebacterium* sp, *Enterobacter cloacae, Salmonella* sp, *Escherichia coli, Shigella* sp, *Bacillus pumilus.* These bacteria species isolated and identified in this study corroborate with those isolated and reported earlier by Okigbo (2005). *Escherichia coli* with 20.83 % frequency of occurrence was the most commonly occurring post-harvest rot inducing bacteria while *Corynebacterium sp* and *Shigella* sp had the least frequency of occurrence with 4.2 % each. The frequency of occurrence base on the different study areas indicates that central market in Bukuru had the highest bacteria species to be 50 % while kugiya and Gyel markets to be 25 % each. The high frequency of bacteria at the central market could be due to poor storage space which increases bruises on the cocoyam tubers.

The Pathogenicity test reveal that most bacteria organism tend to cause severe rot in cocoyam, as observed during the studies. The result reveals that *Enterobacter cloacae* was the most pathogenic bacteria with percentage area of infection of 14.3 %. Pathogenicity test of pure isolates from randomly selected cocoyam cormels, all the bacteria were pathogenic to healthy cocoyam cormels after 10 days under room temperature. The problem of post-harvest losses has been identified as a major constraint to the production of root and tuber corps (Ogali, 1994).

These bacteria inhabit the cocoyam through factors like temperature and relative humidity, which are likely to determine their distribution in the various study areas. The bacteria infect these crops before harvest. Infection could also be cause through injuries after harvest by careless handling or by insects or other animal damage, and also by direct penetration of the intact corms by these pathogenic organisms. Of the factors affecting the quality of cocoyam in storage however, losses caused by microorganisms are by far the most serious (Booth, 1974). In Nigeria, loss of up to 40-50 % cormels due to rot was recorded by the National Root Crops Research Institute (NRCRI, 1980). Ogali (1994) reported 31.6 % and 35.7 % loss after 16 and 24 weeks of storage respectively. Rots of stored cocoyam cormels have been found to start mostly from wounds arising from the point of detachment from the mother corms, harvest bruises or other injuries (Maduewesi and Onyika, 1980). Bikomot and Brecht (1989) reported that losses in cocoyam after harvest manifest in cormel weight loss, sprouting and decay. These interact to affect the quantity and quality of the stored product. But all can be avoided by proper packaging and handling and use of clean planting equipment and healthy planting materials.

CONCLUSION

It is concluded that *Escherichia coli* had the highest frequency of occurrence associated with postharvest rot of *C. esculenta* while *Enterobacter cloacae* was the most pathogenic with highest percentage area of infection. It is recommended that special care should be taken in loading and offloading of cocoyam since bruises serve as infection site for these pathogens and further studies be carried out using molecular identification methods.

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