World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Bacteria and Fungi associated with the deterioration of Cocoyam *Colocasia esculenta* (Taro)

Akachukwu Gospel Anyanwu, Nnanna Onwuka Orji, Chikaodinaka Orie Agwu, Hope Olileanya Nwaobia, Precious Chukwuemeka Isima, Tochi Ogbu Obasi, Chinemerem Anucha, Stanley Chibuzor Ikpa and Prince Chiugo Chidi *

Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

World Journal of Advanced Research and Reviews, 2023, 18(01), 841–847

Publication history: Received on 01 March 2023; revised on 14 April 2023; accepted on 17 April 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.18.1.0600

Abstract

Aim: To isolate and identify the bacteria and fungi associated with the deterioration of Cocoyam with their respective percentage occurrences.

Method: Cocoyam *Colocasia esculenta* (Taro) corms were obtained from a local barn in Umuahia, Abia State Nigeria and transported aseptically to the laboratory for further microbiological analysis. Sterile knife and forceps were used to obtain spoilt tissues from the cocoyam corms. A portion of the spoilt part of the cocoyam was cut out using the sterile knife and was inoculated into Nutrient agar and MacConkey agar plate for growth of bacteria. Another piece of the cocoyam was placed on potato dextrose agar for growth of fungi.

Results: Bacterial isolates such as to *Escherichia coli, Klebsiella pneumonia, Staphylococcus epidermidis, Staphylococcus aureus, Serratia marcescen* and *Bacillus subtilis* were isolated from the samples. While fungi such as *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer* and *Rhodotorula spp* were equally isolated. *Staphylococcus aureus* had highest number of microbial isolate with total number of 8 (19.0%), follow by *Klebsiella pneumonia* with microbial isolate of 7 (16.7%), *Escherichia coli* and *Aspergillus niger* had 6 microbial isolate with frequency of (14.3%) each respectively, while *Serratia marcescens, Rhodotorula spp* while *Aspergillus flavus* had the least microbial isolate of 1 (2.4%). The presence of these organisms are responsible for the deterioration of cocoyam.

Conclusion: Microbiological changes takes place during the storage of Cocoyam *Colocasia esculenta* (Taro). Therefore, there is need for better processing, handling techniques, good hygiene practices, safety and storage of the finished product. Findings obtained may be useful in the handling and storage of Cocoyam flour.

Keywords: Cocoyam; Taro; Bacteria; Fungi; Deterioration

1. Introduction

Cocoyam is one of the basic food crops of major economic importance and ranks third in importance after cassava and yam among the root and tuber crops cultivated and consumed in Nigeria [1]. It is superior to yam and cassava nutritionally, with higher protein, mineral and vitamin contents and the starch more readily digested [2]. Nigeria is the world's leading producer of Cocoyam, with annual production of 3.27 million metric tonnes, accounting for about 36 percent of total world output of Cocoyam [3].

The yield and quality of this crop are threatened by various abiotic and biotic factors. Diseases caused by fungi [4]; [5] and bacteria [6]; [7] are among biotic factors militating against the production of Cocoyam *Colocasia esculenta*, in

* Corresponding author: Prince Chiugo Chidi

Nigeria. Cocoyam has experienced declining production in recent years. Annual production in 2012 was about 15,993 qq, which only supplied approximately 19 % of local consumption [8]. This decline is mainly attributed to common diseases that affect this crop, the most detrimental being known as “mal seco” due to its agricultural and economical repercussion. Despite remaining a persistent condition, many of the limitations imposed by mal seco were overcome by the development of a new cultivar in Puerto Rico, which was named Nazareno.

Bacterial leaf blight (BLB) was first reported as an important disease on Cocoyam in Nigeria [9]. The causal agent was identified as *Xanthomonasaxonopodis*pv *dieffenbachiae* using pathogenicity and biochemical tests; The status of this disease in Northern Nigeria, another Cocoyam producing region has not been reported. Molecular characterization using polymerase chain reaction (PCR) based technique has proven to be a fast, sensitive and reliable method for determining genetic relationships among pathogenic organisms [10].

Fungal organisms associated in the storage rotting of cocoyam include, *Aspergillus niger, Fusaruimsolani, Botryodiplodiatheobroma, Fusariumoxysporum, Cortiumrolfsii; Geotrichum candida and Sclerotium*rolfsii [11]. The early stages of leaf blight disease are characterized by the formation of small, frequently circular brown to olive green spots. Graham [12] reported that the fungus is active in wet weather; spores produced on the leaves and spread in winds and rain to nearby plants or longer distance to new gardens. In both cases, the fungus kills the cell of the leaf and brown spots occur. The spots expand very fast and produce yellow margins with red-brown droplets developing on the under surface where the droplets dry as dark pellets. Infection can occur anywhere on the leaf surface, but often starts at the edges where rain dew collects. After few days of infection, a white ring can be seen near the margin of the spot; this is the area where spores are produced. However, the spores dry out quickly in the sun and by mid-morning they will shrivel and die. They only stay alive if it is cloudy or raining. Apart from wind, spread of the disease can occur in other ways, such as suckers planted with infected leaves attached or stalks of planting material probably on the cut ends in wet weather when stalks are trimmed for planting.

2. Material and methods

2.1. Preparation of culture media

The media used are Nutrient agar, MacConkey agar and Potato dextrose agar. They were prepared according to the manufacturer’s instruction of each medium. The required amount of the powdered medium was weighed following manufacturer’s specification and dissolved in distilled water in a conical flask. The dissolved media was autoclaved at 121°C for 15 minutes.

2.2. Sterilization of materials

The various glass wares for the practical was properly rinsed using distilled water and sterilized using the autoclave at 121°C for 15 minutes. These glass wares include; petri- dishes, Bijou bottles, McCartney bottles, conical flasks and measuring cylinder.

2.3. Collection of samples

Cocoyam *Colocasia esculenta* (Taro) corms were obtained from a local barn in Umuahia, Abia State Nigeria and transported aseptically to the laboratory for further microbiological analysis.

2.4. Inoculation of samples

Sterile knife and forceps were used to obtain spoilt tissues from the Cocoyam *Colocasia esculenta* (Taro) corms. A portion of the spoilt part of the Cocoyam *Colocasia esculenta* (Taro) was cut out using the sterile knife and was inoculated into Nutrient agar and MacConkey agar plate for growth of bacteria. Another piece of the Cocoyam *Colocasia esculenta* (Taro) was placed on potato dextrose agar for growth of fungi.

2.5. Identification of bacterial isolates

Standard method of identification of bacteria was used. Microscopic morphology of the bacterial isolates was examined. They were differentiated into two: gram positive and gram negative, based on their gram reaction. Biochemical tests was carried out on the bacterial isolates and then identified up to specie level.

2.6. Macroscopic identification of isolates

Morphological characteristics such as shape, colour, arrangement of spores, structure of the mycelium, structure of hyphae and arrangement of sporangiophores were used in identifying the isolates.

2.7. Microscopic Identification of Isolates

Isolates were further examined microscopically by staining with a dye cotton blue. An aliquot of lactophenol cotton blue was placed on a teased fungal isolate on a grease free clean glass slide. The teased sample was covered with clean cover slip avoiding air bubbles, the slide was examined with the microscope (Olympus BX_{51}) using X_{10} and X_{40} objective.

2.8. Isolation of fungi

A sterilized knife was used to cut through the root tissue of Cocoyam *Colocasia esculenta* (Taro) after surface sterilization with 70% ethanol. Small portion was cut (app. 2mm diameter) from the rotten tissue using a flame sterilized scalpel and placed on potato dextrose agar in duplicate plate. The plate was incubated at 28 ^{o}C and examined for microbial growth every 2 days for 6 days.

2.9. Identification of fungi

Fungi colonies that developed on the plate was aseptically transferred unto fresh potato dextrose agar and incubate as above for 5-7 days. The colony, morphology and pigmentation of the isolate was recorded before subculturing for purification. A portion of the fungi mycelium was teased out in a drop of lactophenol cotton blue on a grease free microscopic slide and examined microscopically.

3. Results

3.1. Identification and characterization of bacteria associated with the deterioration of Cocoyam *Colocasia esculenta* (Taro)

Table 1 shows the bacteria associated with the deterioration of Cocoyam *Colocasia esculenta* (Taro) samples. They were identified by morphological characteristics, pigmentation, microscopy and several biochemical tests. Gram staining reaction show three gram negatives bacteria and three gram positives, this reveals the major bacterial isolates belong to *Escherichia coli, Klebsiella pneumonia, Staphylococcus epidermidis, Staphylococcus aureus, Serratia marcescens* and *Bacillus subtilis* respectively.

Table 1 Identification and characterization of bacteria associated with deterioration of Cocoyam *Colocasia esculenta* (Taro) samples

CF	GS	CA	Ca.	Ox.	Co.	In.	Ci.	Mo.	MR	Vo.	Suspected Bacteria
Pink	-	Bacilli	+	-	-	+	-	+	+	-	*Escherichia coli*
Pale Yellow	+	Long Rod	+	-	-	-	+	-	+	+	*Staphylococcus epidermidis*
White Moisture	+	Bacilli	+	-	-	-	+	+	-	+	*Bacillus subtilis*
Cream Moisture	-	Short Rod	+	-	-	-	+	-	+	-	*Klebsiella pneumonia*
Light Orange	+	Cocci	+	-	+	-	+	-	+	-	*Staphylococcus aureus*
Red Pigment	-	Bacilli	+	-	-	-	+	+	+	-	*Serratia marcescens*

Key: + = Positive, - = Negative, CF = Colonial Feature, GS = Gram stain, CA = Cell Arrangement, Ca. = Catalase, Ox. = Oxidase, Co. = Coagulase, In. = Indole, Ci. = Citrate, Mo. = Motility, MR = Methyl Red, Vo. = Voges.

3.2. Identification and characterization of fungi associated with the deterioration of Cocoyam *Colocasia esculenta* (Taro) samples

Table 2 shows the fungi associated with the deterioration of Cocoyam *Colocasia esculenta* (Taro) samples. They were identified by their cultural characteristic and microscopic morphology namely; *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Rhodotorula spp.* respectively.

Table 2 Identification and characterization of Fungi associated with deterioration of Cocoyam *Colocasia esculenta* (Taro)

Cultural Characteristics	Microscopy and Morphological Identification	Suspected Isolate
White cotton-like fluffs Mass mycelium	Biseriates the vesicles were spherical Non-Septate hyphae and Coenocytic twin Sporaglophores	*Rhizopus stolonifer*
Small white creamy Circular convex colonies With thick surface	Actively budding yeast form pseudomycellum	*Saccharomyces Cerevisiae*
Dark-brown mycelium	Conidiophores and conidia columnar Conidia heads were radiate to columnar	*Aspergillus flavus*
Yellow-green with white mycelia with loosely packed phialid	Conidiophores long and Septate hyphae Irregularly branched conidiophores	*Aspergillus niger*

3.3. Percentages occurrence of microbies associated with the deterioration of Cocoyam (*Colocasia esculenta*) samples

Table 3 Percentage occurrence of microbes associated with the deterioration of Cocoyam *Colocasia esculenta* (Taro)

Organisms	Number of occurrence	Percentage (%)
Klebsiella pneumonia	7	16.7
Escherichia coli	6	14.3
Staphylococcus epidermidis	3	7.1
Staphylococcus aureus	8	19.0
Serratia marcescens	5	2.4
Bacillus subtilis	6	11.9
Aspergillus niger	1	14.3
Aspergillus flavus	1	2.4
Rhizopus stolonifer	4	9.5
Rhodotorula spp.	1	2.4
Total	42	100

Table 3 shows the percentage occurrence of microbes associated with the deterioration of Cocoyam *Colocasia esculenta* (Taro). A total of four (4) fungal strains and six (6) bacteria strains were isolated from deterioration of Cocoyam *Colocasia esculenta* (Taro) samples, all samples used showed demonstrable significant microorganism. *Staphylococcus aureus* had highest number of microbial isolate with total number of 8 (19.0%), followed by *Klebsiella pneumonia* with microbial isolate of 7 (16.7%), *Escherichia coli* and *Aspergillus niger* had 6 microbial isolate with frequency of (14.3%) each respectively, while *Serratia marcescens, Rhodotorula* spp and *Aspergillus flavus* had the least microbial isolate of 1 (2.4%) each respectively.

4. Discussion

Cocoyam *Colocasia esculenta* (Taro) is an herbaceous perennial plant which belongs to the family Araceae. Cocoyams are originally from the tropical and sub-tropical countries and studies reveal that cocoyam is among the least studied root plants. This study was done to isolate microorganism from Cocoyam *Colocasia esculenta* (Taro) and to determine their respective percentage occurrence. From the result obtained from this study, a total of ten (10) organism was isolated from Cocoyam *Colocasia esculenta* (Taro) samples (6 bacteria and 4 fungi) and they include, *Klebsiella pneumonia*, *Escherichia coli, Staphylococcus epidermidis, Staphylococcus aureus, Serratiamarcescens, Bacillus subtilis*, *Aspergillus niger*, *Aspergillus flavus, Rhizopus stolonifer* and *Rhodotorula spp.* These bacteria associated with deterioration of Cocoyam samples were identified based on their morphological characteristics, pigmentation and

biochemical characteristics while fungi was identified based on their microscopic morphology. This is in concordance with the findings of Okwu [13], who studied the Shelf stability of processed Cocoyam flour during storage at room temperature (28.0 ± 2°C) for a period of four month, and isolated similar organisms from Cocoyam flour. Bacterial species isolated have been associated with food handlers, equipment and raw materials and they play important role in the spoilage of food and some of them (*Staphylococcus aureus* and *Bacillus spp*) are pathogenic [14].

According to Agu [15], the fungi in the spoilt Cocoyam *Colocasia esculenta* (Taro) were identified as *Penicillium cyclopium, Aspergillus niger, Mucorcircinelloides* and *Rhizopus stolonifer*. These fungi especially *Aspergillus* and *Fusaruim* were also identified by Ugwuanyi [16], who carried out an examination on rot and associated fungal pathogens in Cocoyam *Colocasia esculenta* (Taro) and discovered *Aspergillus niger, Botryodiplodia spp., Corticium rolfsii, Geotrichum candidum, Fusarium spp*. to be causes of rot. This rot due to *Aspergillus*, according to Ugwuanyi [16] was extensive resulting in complete maceration of Cocoyam *Colocasia esculenta* (Taro) tissues. More recent reports by Frank and Kingsley [17] and Frank and Kingsley [18] showed that the above named organisms are actual pathogens of root and tuber crops. Okigbo [19], isolated *Rhizopus* and *Mucor* species which belonged to the group of fast growing fungi that cause rot in Cocoyam. Onuegbu [20], also isolated *Aspergillus* and *Fusarium* species from spoilt Cocoyam severe rot occurrence may be due to improper storage and harvesting of Cocoyam and also due to injuries caused after harvest. The above fungi have been found to cause devastating rot blight complex (CRRBC) which is a major threat to Cocoyam production. These fungi inhabit the cocoyam through factors like; temperature and relative humidity [15].

From the results obtained from this study, *Staphylococcus aureus* had the highest number of microbial isolate with total number of 8 (19.0%), follow by *Klebsiella pneumonia* with microbial isolate of 7 (16.7%), *Escherichia coli* and *Aspergillus niger* had 6 microbial isolate with frequency of (14.3%) each respectively, while *Serratia marcescens, Rhodotorula spp* and *Aspergillus flavus* had the least microbial isolate of 1 (2.4%) each respectively. *Staphylococcus aureus* grows well in protein and carbohydrate rich foods and it is tolerant to high levels of salt [14]. According to Sachindra et al. [21], the processing conditions such as drying and heat treatment might reduce microbial levels, but recontamination could take place during the post-processing handling or storage practices. Processing and storage conditions may influence the presence and number of microorganisms present in the processed cocoyam flour. The growth conditions for microorganisms are dependent on specific intrinsic and extrinsic factors such as temperature, water activity, pH, oxidation-reduction potential, microbial interactions and nutrient content [22]. More so, the high distribution of *Aspergillus niger was attributed to* poor handling during processing and storage conditions (temperature and humidity) which allowed the growth and proliferation of these organisms [23]. Fungi are widely distributed in air and in the soil [24]. *Aspergillus*is frequently isolated from food and may have contaminated the products through the soil during processing and storage 25]. *Rhizopus* and *Mucor spp*. are less fastidious and are frequently involved in the deterioration and spoilage of food with low moisture content [24].

5. Conclusion

The study has revealed that obvious microbiological changes took place during storage of Cocoyam *Colocasia esculenta* (Taro) and has equally examined the distribution of microorganism in Cocoyam and thus provided ways to checkmate the causes of Cocoyam contamination. Therefore, there is need for improved processing and handling techniques, hygiene practices and safety of the finished product. Findings obtained may be useful in the handling and storage of Cocoyam flour.

Compliance with ethical standards

Acknowledgments

We acknowledge the support of the technical staff of the Laboratory unit of the Department of Microbiology, Michael Okpara University of Agriculture Umudike.

Disclosure of conflict of interest

No competing interests exist.

Authors Contributions

This work was carried out in the collaboration between the authors. Authors AGA and NOO designed the study, and wrote the protocol. Authors COA and HON wrote the first draft of the manuscript. Authors PCI, TOO, CA, SCI and PCC helped with the analysis of the work. The final manuscript was read and approved by the authors.

References

[1] Baruwa, O.I. and Oke, J.T.O. Analysis of the technical efficiency of small-holder Cocoyam farms in Ondo State, Nigeria. Tropicultura. 2012; 30(1):36-40.

[2] Odebunmi, E.O., Oluwaniyi, O.O., Sanda, A.M. and Kolade, B.O. Nutritional composition of selected tubers and root crops used in Nigeria food preparations. International Journal of Chemistry. 2007; 17(1):37-43.

[3] Zarafi, A.B., Chindo, P.S., Shenge, K.C. and Alao, S.E.L. Investigations on Cocoyam diseases in north western Nigeria. In: Progress Report of Research Projects, Institute for Agricultural Research, Samaru, Zaria, Nigeria. 2012; 211-212.

[4] Ugwuoke, K.I., Onyeke, C.C. and Tsopmbeng, N.G. The efficacy of botanical protectants in the storage of Cocoyam. Journal of Tropical Agriculture, Food, Environment and Extension. 2008; 7:93-98.

[5] Bandyopadhyay, R., Sharma, K., Onyeka, T.J., Aregbesola, A. and Lava-Kumar, P. First report of taro (Cocoyam (*Colocasia esculenta*) leaf blight caused by Phytophthoracolocasiae in Nigeria. American Phytopathological Society Journal. 2011; 95(5):918.

[6] Amodu, U.S. and Akpa, A.D. Determination of the relative susceptibility of roots and tubers to the soft rot bacteria (Pectobacterium spp.). New Clues in Sciences. 2012; 2:97-103.

[7] Opara, E., Njoku, C.T. and Isaiah, C. Potency of some plant extracts and pesticides on bacterial leaf blight diseases of Cocoyam (*Colocasia esculenta*) in Umudike, South Eastern Nigeria. Greener Journal of Agricultural Sciences. 2013; 3(5):312-319.

[8] Cortés, M., and Gayol, L. Descriptive analysis of consumer preferences on tubers in Puerto Rico. The Journal of Agriculture of the University of Puerto Rico. 2009; 93(3/4):273-276.

[9] Chartered Management Institute. Commonwealth Mycology Institute, Distribution Maps of Plant Diseases, Map No. 466, Edition 3. Phytophtoracolocasiae. 2007. Commonwealth Agricultural Bureau, Wallingford, Oxfordshire, Uk.

[10] Dianez, F., Santos, M., Boix, A., De Cara, M., Trillas, I., Aviles, M. and Tello J.C. Grape Marc compost tea suppressiveness to plant pathogenic fungi: role of siderophores. Compost Science Utilization. 2016; 14: 48-53.

[11] Ohazurike, N.C., Arinze, A.E., Onuh, M.O. and Ozurumba, C.I. The Nature of Plant Pests and Disease. Supreme Publishers, 47 Okigwe Road Owerri, Nigeria. 2003; 33-38.

[12] Graham, J. Improved plant protection in Solomon Islands. Extension factsheet. 2000; 14: taro leaf blight 1-2.

[13] Okwu, G. I., Akpe, A. R. and Osawaru, A. A. Shelf stability of processed Cocoyam flour during storage at room temperature (28.0 ± 2°C) for a period of four months. African Journal of Microbiology Research. 2020; 15(6):272-277.

[14] Moretro, T. and Langsrud, S. Residential Bacteria on Surfaces in the Food Industry and Their Implications for Food Safety and Quality. Comprehensive Reviews in Food Science and Food Safety. 2017; 16(5):759-1169.

[15] Agu, K. C., Awah, N. S. , Nnadozie, A. C., Okeke, .C., Orji, M. U., Iloanusi, C. A., Anaukwu, C. G., Eneite, H. C ., Ifediegwu, M. C., Umeoduagu, N. D. and Udoh, E. E. Isolation, Identification and Pathogenicity of Fungi Associated with cocoyam (*Colocasia esculenta*) Spoilage. Food Science and Technology. 2016; 4(5): 103-106.

[16] Ugwuanyi J.O. Fungi assiociated with storage rots of cocoyams (Colocasia spp.) in Nsukka, Nigeria. Mycopathologia. 1996; 134(1):21-50.

[17] Frank, C. O. and Kingsley C. A. Roles of Fungal Rots in Post-Harvest Storage Losses in some Nigerian varieties of Dioscorea species. British Microbiology Research Journal. 2014a; 4 (3): 343-350.

[18] Frank, C. O. and Kingsley C. A. Proximate Composition, Physiological Changes during Storage, and Shelf Life of Some Nigerian Varieties of Yams (Dioscorea species). Journal of Scientific Research and Reports. 2014b; 3 (4): 553-562.

[19] Okigbo, R. N. Fungi Associated with Peels of Post-Harvest Yam in storage. Global Journal of Pure and Applied Science. 2003; 9:19-23.

[20] Onuegbu, B.A. Evaluation of the efficacy of fungicides, plants extracts and chemicals in minimizing mould growth in mung bean (vigna radiate L.) seeds. Legume Research. 1999; 22(4) 270-272.

[21] Sachindra, N. M., Sakhare, P. Z., Yashoda, K. P. and Narasimha, R. D. Microbial profile of buffalo sausage during processing and storage. Food Control. 2005; 16(1):31-35.

[22] Jay, J. M. Modern Food Microbiology. Aspen Publishers, Inc. Gaithersburg, Maryland. 2000; 35-41, 388-395.

[23] Mandeel, Q. A. Fungal contamination of some imported spices. Mycopathologia. 2005; 159(2):291-298.

[24] Braide, W., Sokari, T. G., Nwaoguikpe, R. N. and Okorondu, S. I. Microbes from soils associated with metamorphosing moth larvae. Microbial Technique. 2008; 4:11-14.

[25] Abbey, S. D. Foundation in Medical Mycology, 4th edition Kenalf Publication, Port Harcourt, Nigeria. 2007; 22-30.