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FUNGAL AND BACTERIAL PATHOGENS ASSOCIATED WITH SOFT ROT DISEASE OF SWEET POTATO (*Ipomoea batatas,* L. Lam)

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Abstract
This study focused on screening for fungal and bacterial pathogens associated with soft rot disease of sweet potato tuber. Standard microbiological techniques were used to characterize and identify the fungi and bacteria isolated. The fungal and bacterial isolates were subjected to pathogenicity test to ascertain their degree of pathogenicity. *Aspergillus flavus, Botryodiplodia theobromae*, *Fusarium oxysporium* and *Erwinia spp* induced rots in the healthy sweet potato tubers at significant levels, with rot length ranging from 12.40 ±0.125mm to 15.25±0.135mm; an indication of being pathogenic. *Rhizopus stolonifer, Bacillus spp* and *Corynebacterium spp* with rot length 7.63±0.250mm, 4.63±0.145mm, and 4.50±0.157mm respectively, were not pathogenic. The fungal isolates pre-dominated the bacterial isolates as pathogens associated with soft rot disease in the sweet potato tubers. This study therefore provide a benchmark upon which future research can be carried out in the quest to reduce post-harvest loss of sweet potato tubers.

Keywords: Soft rot disease, sweet potato, fungal and bacterial pathogens

Introduction

Sweet potato (*Ipomoea batatas,* L. Lam) belongs to the family of Conovolvulaceae and genus *Ipomoea.* It is a tuber that is still cultivated in many countries today (Hu *et.al.,* 2004). Sweet potato is among the world's most vital, versatile and underutilized food crop that ranks fourth after rice, yam and wheat (Low *et al.*, 2009). It is appreciated as alternative food crop and a security crop in case of famine in most African countries because of its fast growing period, low inputs and work requirement. Sweet potato tubers are susceptible to soft rot disease caused mainly by fungi such as *Botryodiplodia theobromae, Cerato cystis, Rhizopus oryzae, Aspergillus flavus, Fusarium solani and Sclerotium rolfsii*, (Ray and Ravi, 2005, Agu *et al.,* 2015). Although, few bacteria like *Erwinia*, *Bacillus*, *Flavobacteria* have been reportedly associated with sweet potato tuber soft rot disease (Umunna and Agugo, 2014; Oduola *et al.,* 2018). There is therefore need to identify and control these pathogens to mitigate the post-harvest loss of sweet potato tubers.

Materials and Methods

Samples

Healthy and rotten (infected) sweet potato tubers were collected from Sweet Potato Programme of National Roots Crop Research Institute (NRCRI), Umudike, South-East, Nigeria.

Isolation of fungi and bacteria from infected sweet potato tubers

Isolation of fungi and bacteria from the rotten sweet potato tubers was carried out by standard microbiological culture technique adapted by Agu *et al.* (2015) with slight modification. The rotten sweet potato tubers washed with running distilled water were surface sterilized with 70% ethanol and cut open. About 3mm diameter of the infected tissues were picked with a flamed sterilized forcept and inoculated in solidified Sabouraud Dextrose Agar (SDA) and Nutrient Agar (NA) medium. Each inoculum was placed on the surface of the solid medium and spread evenly on the plate with the aid of a sterile bent glass rod. The inoculated NA plates were incubated at 37°C for 24 hours at room temperature for 2-7days for the SDA plates. Each colony were sub-cultured on freshly prepared SDA and NA medium to obtain pure culture of the fungal and bacterial isolates respectively. The pure cultures of fungi and bacteria were inoculated in slants of SDA and NA respectively. These slants were stored in the refrigerator at 4°C.

Characterization and identification of fungal and bacterial isolates

Characterization and identification of fungal isolates were carried out according to standard microbiological

method adapted by Anukworji *et al.* (2013). Texture, extent of growth, colour and presence of visible mycelia, pigmentation, shape and edge of each colony were studied and documented. Slide mounts of each isolate were prepared and stained with lactophenol or cotton-blue dye, then examined under a low power microscope (x20) for presence of sporogiophores, conidiophores, direction of growth, branching, septation, shape and colour of conidia and spores, among others. These characteristics were recorded and compared with those of Practical Laboratory Mycology manual by Koneman and Glenn (1985) for the identification of all the fungal isolates. The bacterial isolates were subjected to series of biochemical tests and identified according to Cheeseburg (2005).

Preparation and standardization of fungi isolates

This was carried out according to standard microbiological method adapted by Petrikkou *et al*. (2001). A loopful of the pure fungal isolates grown on SDA plate were each aseptically transferred into 5ml of distilled water using a sterile wire loop. The inoculum was transferred to a sterile syringe attached to a sterile filter of pore diameter 11$^{\mu m}$ (Millipore, Madrid, Spain). The suspension for each inoculum were filtered and collected in a sterile tube. The filtrate containing mainly spores were serially diluted to 10^{-6}, then adjusted to contain inoculum size between 1.0×10^{6} and 5.0×10^{6} spores/ml by direct microscopic enumeration using a cell counting hematocytometer (Neubauer-chamber Merck S.A., Madrid Spain). All adjusted suspensions were plated on SDA, incubated at room temperature and observed daily for growth. The colonies were counted as soon as possible after the observation of visible growth.

Preparation and standardization of bacterial isolates

The bacterial isolates were standardized based on standard microbiological method adapted by MAST (2005). Each isolate was diluted with sterile physiological saline solution to achieve an inoculum concentration of approximately 10^{6} cfu ml^{-1} with reference to 0.5 McFarland standard prepared by transferring 0.5ml of 1.0%w/v $BaCl_2$ into 99.5ml of 1% v/v H_2SO_4. Then, confirmed by further plating on nutrient agar.

Pathogenicity test of isolates

Pathogenicity test of the isolates was carried out based on standard microbiological method described by Anukworji *et al.* (2013) with slight modification. Healthy sweet potato tubers were marked and surface disinfected by swabbing with 70% ethanol solution. Carefully, with the aid of a flamed cock borer of 5mm diameter, holes were bored on the healthy sweet potato tubers and the cylindrical flesh removed and kept under cover in sterile petri dishes. Then using flamed cock borer of 3mm diameter, circular discs were cut out solid medium containing standard culture [$(1\text{-}5)\times10^{6}$ spores/ml] of each isolate and inoculated into the bored hole of the sweet potato tuber and covered with the flesh removed from that particular hole (care was taken to ensure that the activity was completed in the shortest possible time ≤1min to avoid possible contamination by microorganisms from the atmosphere. A control experiment was set up in which the bored hole was left without inoculation, labeled accordingly and allowed to incubate at ambient temperature for 7-14 days, and were observed daily for signs of rot including softening, discoloration, offensive odor , drying up of flesh etc. at the end of the incubation period. The sweet potato tubers were cut open carefully along the line of inoculation to expose the inner parts which were examined for rots. The length of rot was recorded thus: 0.0.00-9.00mm=no rot, 9.00-14.00mm=2 rots, 14.00-21.00mm=3 rots, >21.00mm=4 rots. The isolates that caused rots were considered pathogenic, while those that don't, were considered not pathogenic.

Results and Discussion

The following isolates namely; *Aspergillus flavus*, *Fusariums oxysporium*, *Rhizopus stolonifer*, *Botryodiphidia theobromae, Aspergillus flavus, Erwinia spp., Bacillus spp. and Corynebacterium spp*. were present in the infected sweet potato tubers investigated as shown in Tables 1 and 2. The frequency of occurrence of the fungal isolates (Table 3) show that *Aspergillus flavus* predominated over all the isolates with percentage occurrence of 100%. *Fusarium oxysporium* recorded 85.7% and 71.45 for *Rhizopus stolonifer* and *Botryodiphidia theobromae* each. *Erwinia spp had* 71.4%, *Bacillus spp.* 57.1% and *Corynebacterium spp.* 28.6% .

The pathogenicity test results as seen in Table 4 show varying levels of rots caused by the isolates on the healthy sweet potato tubers with significant differences ($p<0.05$) in the rot length. *Aspergillus flavus, Fusarium oxysporium, Botryodiplodia theobromae*, and *Erwinia spp* significantly induced rots on the healthy sweet potato tubers with rot length ranging from 12.40 ±0.125mm to 15.25±0.135mm; an indication of being pathogenic. *Rhizopus stolonifer, Bacillus spp* and *Corynebacterium spp* with rot length 7.63±0.250mm, 4.63±0.145mm, and 4.50±0.157mm respectively were not pathogenic. The abundance of fungal over the bacterial isolates as pathogens responsible for sweet potato soft rot disease was not a coincidence as several studies have revealed the occurrence of fungi than bacteria associated with sweet potato soft rot disease (Ray and Ravi, 2005, Suleiman and Falaiye, 2013, Oduola, *et al.,* 2018). Hence, measures should be taken to control the spread of these pathogens to reduce post harvest loss of sweet potato tuber in our farms.

Fig1: Healthy sweet potato tuber

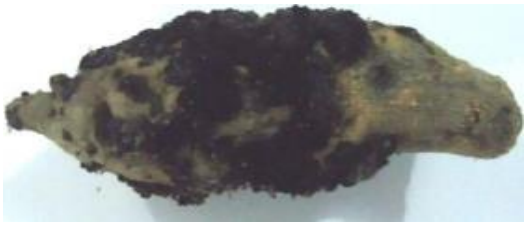

Fig 2: Rotten sweet potato tuber

Fig 3: Morphology of *R. stolonifer* on SDA

Fig 4: Microscopy view of colony of *R. stolonifer* x20

Fig 5: Morphology of *A. flavus* on SDA

Fig 6: Microscopy view of colony of *A. flavus* x20

Fig 7: Morphology of *F. oxysporium* on SDA

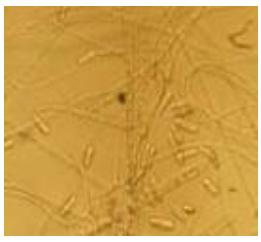

Fig 8: Microscopy view of colony of F. *oxysporium* x20

Fig 9: Morphology of *B. theobromae* on SDA.

Fig 10: Microscopy view of colony of *B. theobromae* x20

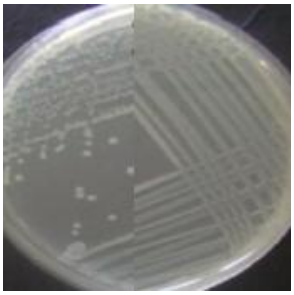

Fig 11: Pure culture of *C. spp* on NA

Fig 12: Pure culture of *B. spp* on NA

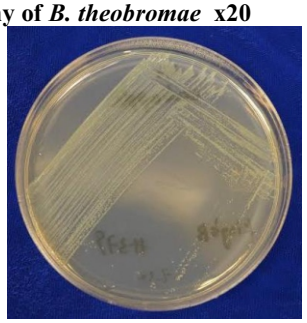

Fig 13: Pure culture of *E.spp* on NA

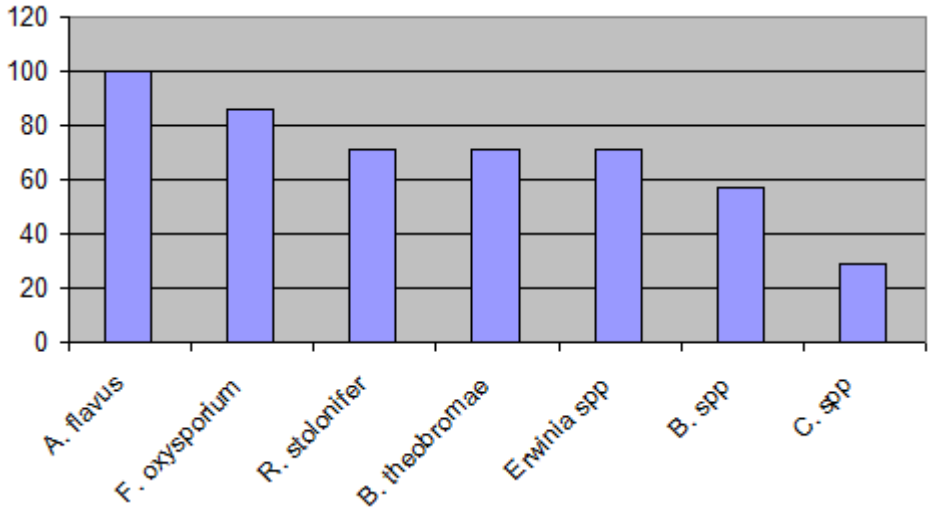

Fig 14: Percentage occurrence of isolates in rotten sweet potato tubers

Table 1: Characterization and Identification of fungal isolates

Colonial characteristics	Microscopic characteristics	Isolates
On SDA, colonies are moderate to rapid growing, flat, covered by a dense layer of yellow-greyish green conidial heads.	Septate hyphae with Conidiophores were hyaline, yellow-greyish green in colour, erect, simple, with foot cells basally inflated at the apex forming globose to subglobose vesicles. Presence of cleistothecia fruiting body. Phialides are in two series covering nearly entire vesicle.	*Aspergillus flavus*
Colonies are woolly to cottony, flat, fast growing, from the front, the colour was white and from the reverse side it was dark purple on SDA	Phialides are cylindrical, with component of a complex branching system. Macroconidia were produced from phialides on branched conidiophores and have a distinct basal foot cell and pointed distal ends.	*Fusarium oxysporium*
Colonies are velvety and fast growing, with shades of green on SDA, cottony to fluffy, white to yellow, becoming dark-grey.	Sporangiospores are hyaline, grey or brownish, globose to ellipsoidal, and smooth-walled, and erect, branched, forming large, terminal, globose to spherical, multispored sporangia, without apophyses and with well-developed subtending columellae.	*Rhizopus stolonifer* *Botryodiphidia theobromae*
Colonies are compact with dense layer of black conidial heads	Conidia are dark ovoid to elongated and are found in pycnidium. Presence of branched mycelia of various length and size.	

Table 2: Characterization and identification of bacterial isolates

Isolate	Catalase test	Oxidase test	Sporulation on nutrient agar	Gelatin hydrolysis	Starch hydrolysis	Lecithin hydrolysis	Gram test
Bacillus spp	+	-	+	+	-	+	+
Corynebacterium spp	+	-	-	+	-	+	+
Erwinia spp	+	+	-	+	+	-	-

+ = positive, - = negative , · = not carried out

Table 3: Frequency of occurrence of isolates in rotten sweet potato tubers

Isolates	Number of isolates	Frequency of occurrence (%)
Aspergillus flavus	7	100.0
Fusarium oxysporium	6	85.7
Rhizopus stolonifer	5	71.4
Botryodiphidia theobromae	5	71.4
Erwinia spp	5	71.4
Bacillus spp	4	57.1
Corynebacterium spp	2	28.6

Table 4: Pathogenicity tests of isolates

Isolates	Mean rot length (mm)
Control	4.20 ±0. 150
Aspergillus flavus	15.25±0.135
Fusarium oxysporium	12.40 ±0.125
Rhizopus oxysporium	7.63±0.250
Botryodiplodia theobromae	13.45 ±0.145
Erwinia spp	12.50 ±0.125
Bacillus spp	4.63±0.145
Corynebacterium spp	4.50±0.157

p<0.05, 0.00-9.00mm=no rot, 9.00-14.00mm=2 rots, 14.00-21.00mm=3 rots, >21.00mm=4 rots

Conclusion

Soft rot disease in sweet potato tuber could be caused by pathogens like *Aspergillus flavus, Botryodiplodia theobromae*, *Fusarium oxysporium* and *Erwinia spp* which are predominantly fungi. The fungi isolated in this study showed significant levels of pathogenicity than the bacterial isolates. This study therefore provides a benchmark for future studies to mitigate post-harvest loss of sweet potato tubers.

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