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Characterization and Identification of Fungi Associated With the Spoilage of Potato Tubers Sold At Rumuokoro and Mile 3 Market

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Abstract: Major fungal diseases affecting Irish and Sweet potato purchased from Rumuokoro and Mile 3 Markets were evaluated. The Potato tubers were selected at random and analyzed using Standard bacteriological plate count method and fungi isolated and identified using cultural methods. The microbial count of fungi isolated and identified from Irish potato obtained from Rumuokoro market, ranged from $1.65x\ 10^5$-$5.3x10^5$ with sample P1 was having the highest count and sample P3 having the lowest counts. The microbial count of fungi isolated from Irish potato obtained from Rumuokoro market ranged from $1.95x10^5$-$7.25x10^5$ with sample P5 having the highest count and sample P8 having the lowest count as shown. The analysis showed that there is significant difference of the fungi isolated from Sweet and Irish potato isolated from Rumuokoro with P-value of $5.6x\ 10^5 \pm 1.0x10^5$ and $5.14\ x10^5 \pm 10^5$. There is also significant difference in the Irish Potato and Sweet potato isolated from Rumuokoro market with P-Value of 5.6X $10^5 \pm 1.59x10^5$ and $3.62x10^5 \pm 1.03x10^5$ at 24 degree of freedom. Molds associated with spoilage of Sweet and Irish potato tubers in Mile three and Rumuokoro markets in Obio-Akpor Local Government areas of Rivers State, were *Rhizopus sp.* Aspergillus niger and *Aspergillus fumigatus, Penicillium sp.* and *Yeast sp.* The result obtained shows that the Percentage of occurrence of fungi isolated from Irish potato ranged between 9% to 36% with *Aspergillas niger* having the highest parentage of occurrence. Followed by *Yeast sp* which had a percentage occurrence or 27%. *Aspergillus flavus* and Penicillium sp showed the lowest percentage of occurrence of 19% each. The result obtained shows that the Percentage of occurrence of fungi isolated from Sweet potato ranged from 9% to 36% with *Aspergillus niger* having the highest percentage of occurrence. Aspergillus Sp. Rhizopus sp showed the lowest percentage occurrence of 9%. It was perceive that the most frequently isolated fungus from spoilt sweet potato tubers in the markets examined were *Rhizopus stolonifer* and *Aspergillus niger*.

Keywords: Irish potato, sweet potato, Aspergillus niger, Rhizopus stolonifer,Penicillum sp, Yeast sp.

INTRODUCTION

Root crops are largely produced in the humid equatorial areas and the sub humid savannas adjacent to the equatorial zone (Aimienyo and Ataga, 2007). The Irish potato and Sweet potato, although native to highland equatorial tropics, has been developed most successfully as temperate crop and is recently returning to the tropics. The yield of these crops has increased immensely recently and has surpassed yam and sweet potato together, doubling in the last decade. Much of this increase has been in highland tropics, not only in the aborigin of the crop in South America but also countries such as Kenya, India and Nigeria (Ogbo and Agu, 2014).

The work of Mauseth, (2012) showed that stem tubers are normally off shoot organs springing out from branches off a mature plant. The offspring or new tubers are attached to a parent tuber or form at the end of a (initiated below ground) rhizome, a very simple example is Yam.

The work of McGill *et al*., (2013) showed that Potatoes provide major nutrients to the diet including vitamin C, potassium, and dietary fiber. As a matter of fact, potatoes have a more suitable overall nutrient-to-price ratio than any other vegetables and an important staple worldwide (IPC, 2018).

Agwu *et al*., (2015) showed that Sweet potato is a root crop cultivated in many countries including Nigeria, Sierra Leone, and Ghana. Sweet potato can be said to be an important food crop in Nigeria ranking third amongst important tuber crops of Sub-saharan Africa, after yam which second to cassava as the most valuable tropical root crop and are a staple crop in many parts of Africa and Southeast Asia (Ogbo and Agu, 2014). Sweet potato is grown in general for its storage roots, which are eaten fresh, steamed, or boiled, often the leaves are eaten as vegetables or may be converted into flour or starch, and the vines are fed to livestock (Hu *et al.*, 2004). During harvest, sweet potato roots are delicate products and large number are lost during transportation, storage and selling. Potato roots have high water content giving rise to storage problems and also exposed to microbial attacks, resulting in huge amount of losses. Normally the fungi causing rot in sweet potato are lesion pathogens (Rees *et al.*, 2003). According to the work of Agwu *et al*., (2015) they showed that some of the various rots seen in potato include; Dry rot (*Aspergillus niger, Aspergillus fumigates*), Black rot (*Ceratocystis fimbriata*), Foot rot (*Plenodomus destruens*), Soft rot (*Rhizopus stolonifer*), and Blue mold rot (*Penicillium spp*), Fusarium root and stem rot (*Fusarium solani*.).

sweet potato roots can be stored for several months, Under a supervise environment, traditional storage method which include leaving tubers on open floor and burying in the ground, has recorde rodent destructions, d heavy losses owing to sprouting, and insect and microbial damage (Ogbo and Agu, 2014). Sweet potato may be easy to grow and eaten by many, it's after harvest has not been taken care of properly. This

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especially is applicable to storage by small scale farmers, although this group of producers is the one that cultivates and stores roots and tubers most remarkably in Africa (Ogbo and Agu, 2014).

Irish potato is a complete food that is high in carbohydrate, protein and vitamins contents, it also contains some minerals such as potassium, phosphorus, iron and magnesium and has about 70% water (Murano, 2003). Irish potato could be said to be brown and oblong in shape, it has a green colouration when not fully matured and turns yellowish brown when it gets fully matured. It matures on the sub surface and develops from the swollen, underground stem of the plant. It grows well weather where nights are cold with warm day normally when the tubers are forming. Temperature of $15^{o}C - 20^{o}C$ is considered best for the tuber growth. In view of its nutrient and water contents, it is easily taken over by microorganisms especially when the skin is physically damaged due to injury during harvest which normally occurs under humid condition (Donna, 2008).

Various factors could cause the spoilage of various foods including Irish potato (Istifanus *et al.,* 2014). The causes of decay of potato could come from physical damage as a result of water loss. It could also happen as a result of long storage in the soil where it is indirect contact with soil microorganisms and the soil is known to be the reservoir of biodeteriogens. Some tuber diseases such as dry rots appear mostly in storage while others such as soft rot affect tubers at every stage namely in field storage and in transit and may lead to a lot of potato loss under varying conditions.

Fungi associated with this type of rot are *Rhizopus* spp, *Mucorcircinelloides*, *S. rolsii*, and *Rhizoctonia solani* and *Armillariella mell*ea. Ataga and Amienyo, (2007) reported that in South western, Nigeria *Rhizopus stolonifer* is the most common isolated fungus from spoilt sweet potato tubers. Agu, (2014) reported that fungi constitute a big problem in storage of many agricultural commodities.

MATERIALS AND METHODS

Sample Collection

A variety of Sweet potato and Irish potato cultivated in South South Nigeria was used in the study. A total of 60 tubers were obtained from Mile 3 and Rumuokoro markets in Obio-Akpor Local Government of Rivers State. The spoilt potato tubers were packed in sterile cellophane bags and transported to the laboratory of the Department of Microbiology Rivers State University and was used for the study.

Isolation of Rot-Causing Fungi

Spoilt potato tubers were washed in distilled water and were opened up with a sterile knife. 50g of the diseased tissues were weighed from the point of advancement of rot with the aid of a sterile knife and mixed with 250ml of sterile normal saline and was vigorously mixed to form remove the fungi colonies from the potato skin.

Total Fungi Cell Count

The principle of this technique is to determine the total number of viable cells present in samples under examination. The spread plate method was used for this technique.

Test tubes containing 9 ml normal saline (0.09 % w/v NaCl) were arranged in three's and labelled accordingly 1:10, 1:100, and 1:1000). A 1 ml volume of the stock samples was aseptically transferred into the 1:10 labelled test tube. A three ten-fold serial dilution was carried out through the rest of the bottles in the sequence of 1:100 to 1:1000. A 0.1 ml inoculum of the diluted sample of the various concentration of 1:10, 1:100 and 1:1000 were aseptically inoculated into sterile Sabouraud Dextrose agar plate and spread using sterile bent glass rod.Plates were labelled in respect to the dilutions. This was done in duplicate. The plates were incubated in an inverted position at room temperature for 3-7days. Plates were examined after the incubation period and results were recorded.

The presence of colonies on media is indicative of viable cells, and the total viable cell count was determined using a bacterial colony counter.

Identification of Fungi

Identification of fungal isolates was categorically on morphology and microscopy. For fungal identification, a mash of hypha of the test organism was done using Scotch Tape technique.

Microscopy for Fungi

Wet preparations were made by placing the swabs in 10% potassium hydroxide (KOH) mount on a glass slide with cover slip. This was then examined microscopically with x40 objective for the presence of hyphae and arthrospores.

Media Used

Sabouroud Dextrose Agar was used during the analysis. All media were prepare according to the Manufactures direction. 65g of the Sabouroud Dextrose Agar powder was weighed and dissolved in distilled water and mixed properly. The medium was then autoclaved at $121^{O}C$ at 15PSI for 15 minutes. After sterilization, antibiotic was added to the molten agar and mixed properly. 20mls of the media was then poured into sterile Petri dished and allowed to solidify

Characterization of Rot-Causing Fungus

Emphasis of the characterization was based on the description of the morphological appearance of fungal colonies on the Sabouraud Dextrose agar medium and the slide culture technique for microscopic examination with reference to the Manual of Fungal Atlas. For fungal identification, a mash of hypha of the test organism were made on slides containing Lacto phenol cotton blue, covered with a cover slip and observed in X 40 objective of the microscope.

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RESULTS

The microbial count of fungi isolated from Irish potato obtained from Rumuokoro market, ranged from $1.65x10^5$-$5.3x10^5$with sample P11 having the highest count and sample P3 having the lowest count.

Table 1: Total Fungi Plate Count of Irish Potato from Rumuokoro Market

S/N	Samples	Cfu/ml
1	P1	$3.0x10^5$
2	P2	$2.0x10^5$
3	P3	$1.65x10^5$
4	P4	$3.45x10^5$
5	P5	$2.95x10^5$
6	P6	$4.85x10^5$
7	P7	$3.15x10^5$
8	P8	$3.55 x10^5$
9	P9	$3.5 x10^5$
10	P10	$4.2 x10^5$
11	P11	$5.3x10^5$
12	P12	$3.9x10^5$
13	P13	$4.9x10^5$
14	P14	$3.8x10^5$
15	P15	$4.15 x10^5$

The microbial count of fungi isolated from irish potato obtained from Rumuokoro market, ranged from $1.95x10^5$-$7.25x10^5$with sample P5 having the highest count and sample P8 having the lowest count.

Table 2: Total Fungi Plate Count of Sweet Potato from Rumuokoro Market

S/N	**Samples**	**Cfu/ml**
1	P1	$4.55x10^5$
2	P2	$2.5 x10^5$
3	P3	$3.8 x10^5$
4	P4	$5.75 x10^5$
5	P5	$7.25 x10^5$
6	P6	$4.5 x10^5$
7	P7	$3.25 x10^5$
8	P8	$1.95 x10^5$
9	P9	$6.4 x10^5$
10	P10	$4.6 x10^5$
11	P11	$3.9 x10^5$
12	P12	$5.05 x10^5$
13	P13	$3.35 x10^5$
14	P14	$2.3 x10^5$
15	P15	$2.6 x10^5$

The microbial count of fungi isolated from irish potato obtained from Mile 3 market, ranged from $2.5x10^5$-$7.7x10^5$with sample P13 having the highest count and sample P6 having the lowest count.

Table 3: Total Fungi Plate Count of Irish Potato from Mile 3Market

S/N	**Samples**	**Cfu/ml**
1	P1	$3.15x10^5$
2	P2	$6.9 x10^5$
3	P3	$6.0 x10^5$
4	P4	$6.9 x10^5$
5	P5	$5.5 x10^5$
6	P6	$2.5 x10^5$
7	P7	$5.0 x10^5$
8	P8	$5.75 x10^5$
9	P9	$3.0 x10^5$
10	P10	$5.15 x10^5$
11	P11	$6.6 x10^5$
12	P12	$6.8 x10^5$
13	P13	$7.7 x10^5$
14	P14	$6.55 x10^5$
15	P15	$6.75 x10^5$

The microbial count of fungi isolated from Sweet potato obtained from Mile 3 market, ranged from $2.15x10^5$-$7.9x10^5$with sample P11 having the highest count and sample P14 having the lowest count.

Table 4: Total Fungi Plate Count of Sweet Potato from Mile 3 Market

S/N	**Samples**	**Cfu/ml**
1	P1	$6.1x10^5$
2	P2	$7.25x10^5$
3	P3	$4.45x10^5$
4	P4	$2.55x10^5$
5	P5	$6.15x10^5$
6	P6	$3.4x10^5$
7	P7	$7.3x10^5$
8	P8	$2.85x10^5$
9	P9	$3.45x10^5$
10	P10	$6.9x10^5$
11	P11	$7.9x10^5$
12	P12	$7.1x10^5$
13	P13	$6.25x10^5$
14	P14	$2.15x10^5$
15	P15	$3.4x10^5$

Table 5: Macroscopy and Microscopy of Fungi Isolated from Irish Potato

Samples	**Macroscopy**	**Microscopy**	**Suspected Organism**
P1	Black cottony growth, brown radial reverse	Septate hyphae, found head conidia, spore present	*Aspergillusniger*
P2	Black cottony growth, brown radial reverse	Septate hyphae, found head conidia, spore present	*Aspergillusniger*

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P3	Milk colour muccors growth	Coleoid cells	Yeast sp
P4	Green cottony growth, radial periphery act white border	Septate hyphae columnal head, canidia spores	*Aspergillusflavus*
P5	Milk colour muccord growth, brown reverse	Oval shaped cells	Yeast sp
P6	White muccord growth with brown reverse	Long rod-like cells	Yeast sp
P7	Brown cottony growth white periphery and brown radial reverse	Septate hyphae round columinal head spores	*Aspergillusniger*
P8	Black cottony growth brown radial reverse	Septate hyphae found head conida, spore present	*Aspergillusniger*
P9	Green entry growth yellow reverse	Septate hyphae columnar conidia head spore	*Aspergillusflavus*
P10	Green velvety growth with brown reverse	Septate branching hyphae, chain-like conidia, spores	*Penicillium sp.*
P11	Gray fluffy growth, with spots of black	Non septate hyphae, round conidia head	*Rhizopus*

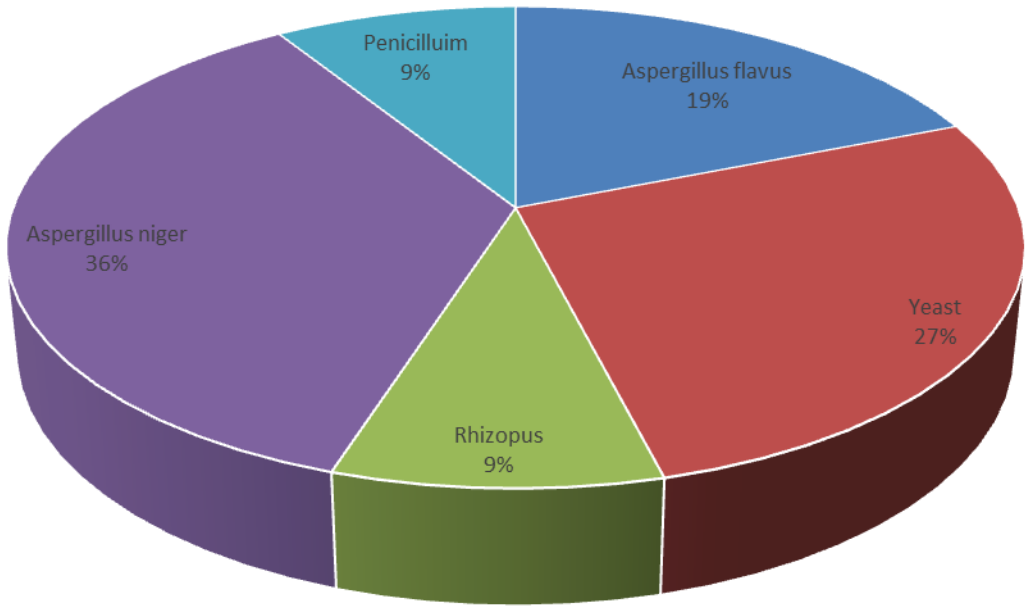

Figure 1: Frequency of Occurrence of Fungi Isolated from Irish Potato

Table 6: Macroscopy and Microscopy of Fungi Isolated from Sweet Potato

Samples	**Macroscopy**	**Microscopy**	**Suspected Organism**
P1	Black cottony growth, brown radial reverse	Septate hyphae, found head conidia, spore present	*Aspergillusniger*
P2	Milk colour muccors growth	Coleoid cells	Yeast sp
P3	Milk colour muccors growth	Oval shaped cells	Yeast sp
P4	Black cottony growth, brown radial reverse	Septate hyphae, found head conidia, spore present	*Aspergillusniger*
P5	Milk colour muccord growth, brown reverse	Oval shaped cells	Yeast sp
P6	Green entry growth yellow reverse	Septate hyphae columnar conidia head spore	*Aspergillusflavus*
P7	Green entry growth yellow reverse	Septate hyphae columnar conidia head spore	*Aspergillusflavus*
P8	Black cottony growth brown radial reverse	Septate hyphae found head conida, spore	*Aspergillusniger*

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		present	
P9	Brown cottony growth white periphery and brown radial reverse	Septate hyphae round columinal head spores	*Aspergillus niger*
P10	Gray fluffy growth, with spots of black	Non septate hyphae, round conidia head	*Rhizopus*
P11	Green velvety growth with brown reverse	Septate branching hyphae, chain-like conidia, spores	*Penicillium sp.*

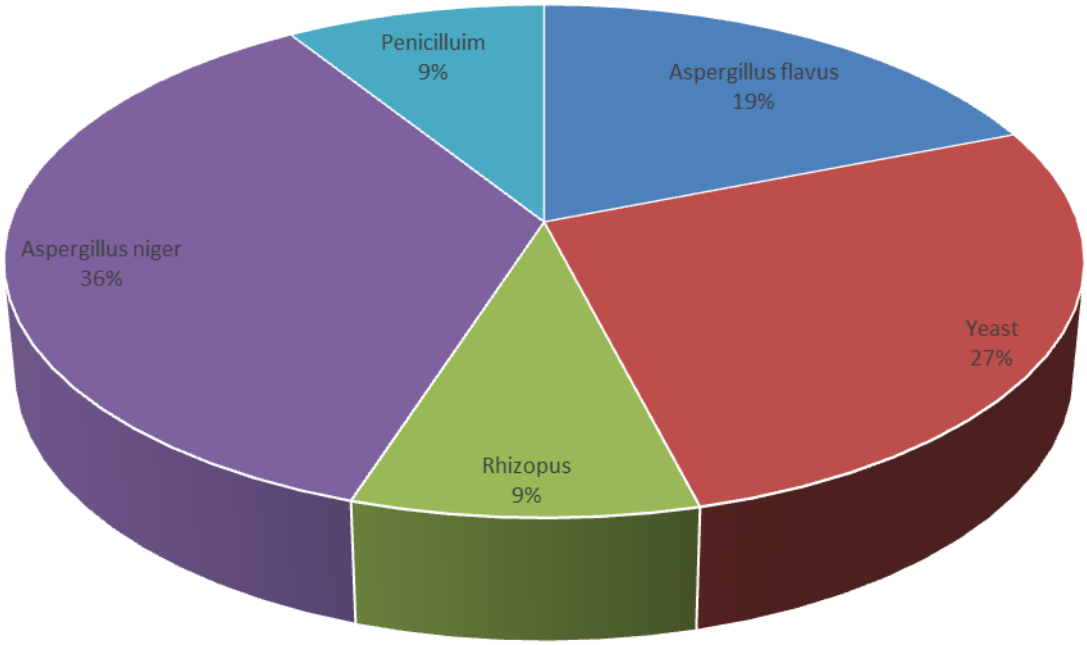

Figure 2: Frequency of Occurrence of Fungi Isolated from Sweet Potato

DISCUSSION

This work has shown that the molds associated with spoilage of Sweet and Irish potato tubers in Mile three and Rumuokoro markets in Obio-Akpor Local Government areas of Rivers State, were *Rhizopus sp, Aspergillus niger and Aspergillus fumigates, Penicillium sp and Yeast sp.* Amienyo and Ataga, (2007) reported that *Rhizopus stolonifer* is the most frequently isolated fungus from spoilt sweet potato tubers in South western, Nigeria. The results of this study are in agreement with the findings of other researchers (Agu, 2014) that fungi constitute a menace in storage rots of many agricultural commodities. It was perceive that the most frequently isolated fungus from spoilt sweet potato tubers in the markets examined were *Rhizopus stolonifer* and *Aspergillus niger*.

Post-harvest rot of Sweet and Irish potato tubers may be due to its low pH, moisture content and nutritional compositions which make it susceptible to infection by fungi. The high incidence of storage rots of sweet potato tubers encountered in Rivers State could be related to prevailing climatic factors and storage conditions. It could also be attributed to handling procedures during harvest, transit, and marketing

Some of the harvesting problems that could cause the injuring of the tubers must have encouraged the microbial colonization of the harvested tubers. Tubers are always in contact with soil after harvest and the soil itself carries with them a lot of biodeteriogens, we can therefore say that soil must have been the major source of microorganisms in these tubers. Soil is known as a hub of both pathogenic and non-pathogenic microorganisms. Several researchers have reported that the numbers of microorganisms in soil habitats normally are much higher than those in fresh water or marine habitats and that bacteria and fungi make up the most ample groups of microorganisms ($3.0 \times 10^6 - 5.0 \times 10^8$) and ($5.0 \times 10^3 - 9.0 \times 10^6$) in respective each soil gram. Istifanus *et al.,* (2014) reported that soil fungi may occur as free-living organisms or in mycorrhizal

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association with plant roots and that they are found mainly in the top 10 cm of the soil and are found below 30 cm infrequently. In the experimental samples microorganisms especially fungi the abundance and distribution indicated that in normal condition of the samples supported the survival of the microbial flora. They grow and carry out active metabolism when conditions are favorable which implies adequate moisture, adequate aeration and relatively high concentrations of utilizable substrates (Oyeyiola and Agbaye, 2013). Species of phycomycetes and other fungal species and yeasts found in this work may not be out of place since these various microorganisms thrive where sugar levels are high . *Aspergillus niger* and *A. flavus*were which are known to produce amylase among the fungi isolates that must have contributed carbohydrate component of the experimental Irish potatoes of the hydrolysis. These fungal isolates also produce proteases, which must have caused the hydrolysis of the protein component of the potatoes and thereby causing decay.

Mold colonization of harvested potatoes may lead to the difficulties seenVV during post-harvest storage of sweet potato.

The analysis showed that there is significant difference of the fungi isolated from Sweet and Irish potato isolated from Rumuokoro with P-value of $5.6x10^5 \pm 1.0x10^5$ and $5.14\ x10^5 \pm 10^5$. There is also significant difference in the Irish Potato and Sweet potato isolated from Rumuokoro market with P-Value of $5.6X\ 10^5 \pm 1.59x10^5$ and $3.62x10^5 \pm 1.03x10^5$.

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