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Species of microorganisms associated with decayed tubers of Irish potato in storage in Plateau State, Nigeria.

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Abstract: *An investigation was carried on species of microorganisms associated with decayed tubers of Irish potatoes in storage in Plateau State, Nigeria. Three batches of decayed potatoes were collected from three different storage depots: Bokkos, Bukuru and Jos. The decayed portions of potatoes from each of the three experimental batches were aseptically cut out from the non-decayed areas of the potatoes with the aid of sterile blades. It was mixed up and further divided into two 2 halves. Each half of the experimental samples was plated out on Nutrient Agar (NA), Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) using serial dilution and soil plate methods. The resultant culture plates were incubated at 25±2°C and examined after 24-48 hours for the development bacteria and yeast colonies and after 4-7 days for the presence of fungal colonies. A total of thirty-nine (39) species of microorganisms made up of eight (8) species of bacteria, three (3) species of yeast and twenty-eight (28) species of fungi were isolated from the decayed potato tubers. The genera that had the highest microbial species included Aspergillus, Lactobacillus and Fusarium. The isolates also contained mesophilic, thermotolerant and thermophilic species. The implications of the results obtained in terms of potato production in Nigeria have been discussed.*

Keywords: *Associated, Bokkos, Bukuru, Jos, Irish potato, Malt Extract Agar (MEA),* m*icroorganisms, Nutrient Agar (NA).*

I. Introduction

Root crops are produced mainly in the humid equatorial areas and the sub humid savannas adjacent to the equatorial zone [1]. The Irish potato, although native to highland equatorial tropics in the Andean altiplano, has been developed most successfully as temperate crop and is recently returning to the tropics. The production of this crop has increased greatly in recent times 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 original home of the crop in South America but also countries such as Kenya, India and Nigeria.

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. It has about 70% water [2]. It is characterized by its large brown oblong shape. It is green in colour when it is not matured and yellowish brown when matured. It grows underground and develops from the swollen, underground stem of the plant. It grows best in climates where cool nights alternate with warm day especially during the period of tuber formation. It requires a growing temperature of $15^{o}C - 20^{o}C$. In view of its nutrient and water contents, it is easily colonised by microorganisms especially when the skin is physically damaged due to harvesting faults which occurs under humid condition as reported by Donna [3].

Food decay is any undesirable change in food that causes it to loose its aesthetic value. Food is also said to be decayed when the original nutritional value, texture and flavour of the food are tempered with through the activities of microbial deteriogens. Generally, decay is the process in which the nutritional constituents of food are hydrolysed to the point that it is no longer acceptable to humans. Various factors could be responsible for the deterioration of various foods including Irish potato. The decay of potato could stem from physical damage as a result of dehydration. It could also happen as a result of long storage in the soil where it is in direct contact with soil microorganisms and the soil is known to be the reservoir of biodeteriogens. Potato decay could also be initiated by mechanical damage caused by insects and rodents. Such mechanical injuries on the potato easily lead to eventual colonisation by microorganisms.

In the developed world, the Irish potato has many industrial and food uses. It could be processed into starch and alcohol [4]. Irish potato is almost exclusively eaten fresh in the developing countries. For nearly 20-30 years the Irish potato was restricted to the highland tropics and was confined to use by privileged economic groups, especially the expatriates. Against this background potato merchants afforded refrigerated storage methods because the production was exceedingly low. This pattern still exists in some areas of the world. Irish potato is fast becoming staple food of many people and countries. Nigeria, Kenya and highland India are of particular interest as the potato is fast becoming quite widely accepted as a subsistence crop as well as a crop for sale at high prices for luxury markets.

However, the wide food and industrial uses of the tuber is hampered by microbial decay problem. Reports of microbial decay of Irish potato in Nigeria are scanty. The present study was therefore designed to find the species of microorganisms associated with decayed potato tubers in storage in Nigeria with a view of sustaining the increasing production rate of the crop.

II. Materials And Methods

2.1 Collection and Processing of Soil Samples

Three batches of decayed potatoes were collected aseptically from Bokkos, Bukuru and Jos storage depots. The samples were put into clean well labelled polythene bags and were transported to the laboratory for processing.

The decayed portions of potatoes from each of the three experimental batches were aseptically sliced out with the aid of sterile blades and further washed in five changes of sterile distilled water to remove surface contaminants. The cut out decayed portions of each batch were mixed up and further divided into two halves. The first half was aseptically reduced into smaller particles and then plated out on Nutrient agar (NA) supplemented with fluconazole 50 mg/ml, Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) supplemented with 2mg/ml gentamycin using the soil–plate method described by Warcup, [5]. The resultant culture plates were then incubated at 25±2^{o}C and examined after 24-48 hours for the development of bacteria and yeast colonies and after 4-7 days for the presence of fungal colonies.

A weight of 10g of the second half of the decayed mixed portions of each batch was also washed in five changes of sterile distilled water and then homogenized with the aid of a clean blender. Serial dilutions (10^{-1}-10^{-5}) of the homogenate were obtained. The diluents were then plated out on Nutrient agar (NA) supplemented with fluconazole 50 mg/ml, Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) supplemented with 2mg/ml gentamycin using serial dilution method described by Durowade *et al*. [6]. The culture plates were incubated at 25±2^{o}C and examined after 24-48 hours for the presence of bacteria and yeasts and after 4–7 days for the presence of fungi.

The colonies of microorganisms that developed were further sub-cultured several times until pure cultures were obtained. The pure cultures of the microorganisms (bacteria and yeast) were gram stained and observed under the microscope for their identifications. They were further subjected to the necessary biochemical tests for the confirmation of their identities. References were made to Lodder [7] and Bergeys manual of systematic Bacteriology, as presented by Madigan *et al*. [8]. The pure fungal colonies that developed were examined under the microscope for their identifications using morphological characteristics. References were also made to stock cultures [9, 10, 11] for the identification of the fungal species.

III. Results

A total of thirty nine (39) species of microorganisms were isolated from the decayed samples of potato tubers of these total microbial isolated. Among the isolates were eight (8) species of bacteria which included *B. stearothermophilus*, a thermophile. Twenty-eight (28) species were fungi while three (3) species were yeasts. Out of the 39 microbial isolates, 29 species representing 33% were isolated from the decayed samples collected from Bokkos and Bukuru respectively, while 30 species representing 34% were isolated from samples collected from Jos. There were close relationships in the species of microorganisms isolated from each of the experimental sites. Fungal species had the highest number (28) among the isolates, representing 71.8%. This was followed by bacteria (8), representing 20.5% and the yeast (3), representing 7.7%. The genus that had the highest species amongst the isolates was *Aspergillus*, with six (6) species. It was closely followed by *Lactobacillus* with five (5) species and *Fusarium* with four (4) species. Out of the 28 species of fungi isolated, seven are thermotolerants, two are thermophilic while the rest are mesophilic in nature. The details of the microbial isolates from the decayed experimental potato tubers are shown in Table 1.

Table 1: Microbial Isolates From The Decayed Potato Tubers

Microbial Isolates	Decayed potatoes from storage Depots			Total
	Bokkos	Bukuru	Jos	
Bacteria				
Bacillus Subtilis	+*	+	+	3
B. Sphaericus	-*	+	+	2
B. Stearothermophilus	+	+	+	3
Lactobacillus acidophilus	+	+	+	3
L. bulgaricus	+	-	-	1
L. delbruekii	+	-	-	1
L. fermenti	+	-	+	2
L. plantarum	+	-	-	1
Fungi				
Absidia corymbifera	+	+	+	3

Alternaria alternata	-	+	+	2
Aspergillus candidus	+	+	+	3
A. fumigatus	+	+	+	3
A. nidulans	+	+	+	3
A. niger	+	+	+	3
A. oryzae	+	+	+	3
A. terreus	+	+	+	3
Aureobasidium pullulans	-	+	+	2
Botrytis cinerea	+	-	+	2
Chaetomium globosum	+	+	+	3
Cladosporium herbarum	-	-	+	1
Curvularia lunata	-	+	+	2
Fusarium moniliforme	+	-	-	1
F. oxysporum	+	+	+	3
F. roseum	+	+	+	3
F. solani	+	+	+	3
Mortierella wolfii	+	-	+	2
Mucor pusillus	-	+	+	2
Myceliophthora thermophilum	+	+	+	3
Paecilomyces variotii	+	+	+	3
Penicillum chrysogenum	+	-	-	1
Rhizopus oryzae	+	+	+	3
R. nigricans	+	+	+	3
Scopulariopsis brevicaulis	-	+	-	2
Syncephalastrum racemosum	+	+	-	2
Trichothecium roseum	-	+	-	1
Ulocladium chartarum	-	-	+	1
Yeasts				
Saccharomyces cerevisiae	+	+	+	3
S. elegans	-	+	-	1
Candida tropicalis	+	+	+	3
Total	29	29	30	88

*+ means present
- means absent

IV. Discussion

The results obtained from the study have shown that a range of microbial species were associated with the decayed potato tubers. Some of the harvest faults that lead to the wounding of the tubers must have facilitated in the microbial colonisation of the harvested tubers. Since the tubers were in contact with soil and the soil particles carried with them loads of biodeteriogens, therefore, soil must have been the source of the decay microorganisms. Soil is recognized as reservoir of microorganisms both pathogenic and non-pathogenic. Several researchers have reported that the numbers of microorganisms in soil habitats normally are much higher than those in fresh water or marine habitats [12, 13] and that bacteria and fungi make up the most abundant groups of microorganisms ($3.0 \times 10^6 - 5.0 \times 10^8$) and ($5.0 \times 10^3 - 9.0 \times 10^6$) respectively per gram of soil. Domsch *et al.* (12) reported that soil fungi may occur as free-living organisms or in mycorrhizal association with plant roots and that they are found primarily in the top 10 cm of the soil and are rarely found below 30 cm. They are most abundant in well-aerated and acidic soils such as the one used in the cultivation of Irish potato. This could explain the increase in the number of fungal species (Table 1) isolated from the decayed Irish potato since the tubers are found at the upper most part of the soil.

The abundance and distribution of the microorganisms especially fungi in the experimental samples indicated that the general condition of the samples supported the survival of the microbial flora. They grow and carry out active metabolism when conditions are favourable which implies adequate moisture, adequate aeration and relatively high concentrations of utilizable substrates [14, 15]. Isolation of species of phycomycetes and other fungal species and yeasts is not surprising since these arrays of microorganisms are sugar lovers. *Aspergillus niger* and *A. oryzae* were among the fungi isolates which are known to produce amylase that must have contributed to the hydrolysis of the carbohydrate component of the experimental Irish potatoes. These fungal isolates also produce proteases, which must have contributed to the hydrolysis of the protein component of the potatoes and its eventual decay. The presence of the species of *Lactobacillus* in the decayed potato tubers means that some of the sugar components of the hydrolysed portion of the carbohydrate constituent of the potatoes must have been converted to lactic acid from the pyruvic acid formed, as all Lactobacilli can easily convert pyruvate to lactic acid.

Paecilomyces variotii, one of the isolates in the present study is known to be weakly xerophilic. The genera of Aspergilli isolated from this research work shares with Penicilli the role of being the most widespread and destructive agent of decay on earth [16]. *Aspergillus* species in general require higher growth temperatures than *Penicillium* and are therefore more commonly found in tropical than temperate zones. That could be the

reason for the isolation of fewer species of *Penicillium* from the decayed tubers of potato. *Aureobasidium pullulans* which was also one of the isolates is a very common decay fungus.

Most species of *Scopulariopsis* are common in soil saprophytic habitats but *S. brevicaulis* is a common biodeteriogen. Smith [17] reported that it flourishes on high-protein substrates such as meat and cheese, and so it was not surprising that it was isolated from decayed Irish potato tubers which contain both essential and non-essential amino acid.

In microbial decay of a food product like Irish potato, the microorganisms may act singly or in groups in the hydrolysis of various complexes of the carbohydrate, protein and other food components to carbon dioxide, water and organic acids. The decay could depend on several factors like the biochemical composition of the tuber, the types of organisms involved, the environmental conditions of both the tubers and the microorganisms together with the changes that occur in the substrate (tuber) during the course of the decay process. The metabolic products that are produced during the decay process impart undesirable odours to the decayed potato and since many microbial metabolites are toxic, the decayed tuber might become a health hazard.

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