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Isolation and Characterization of Soft Rot pathogen of Yam (*Dioscorea spp*) in Nigeria-A Review

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Abstract:

Yam (*Dioscorea spp*) is a tuber crop belonging to the family Dioscoreaceae. The species of economic importance include *Dioscorea rotundata, Dioscorea alata, Dioscorea cayenensis, Dioscorea dumetorum, Dioscorea bulbifera and Dioscorea esculentus.* In West Africa, *Dioscorea rotundata* is also refered to as white yam or white guinea yam. It is the most widely cultivated. It is the most important food and income source for millions of producers, processors and consumers in West Africa. A pathogen or infectious agent is a biological agent that causes disease or illness to its host and it can infect unicellular organisms from all of the biological kingdoms. In the oldest and broadest sense, it is anything that can produce disease, a term which came into use in the 1880s (*"Pathogen". Dictionary.com Unabridged. Random House ; Casadevall, Arturo; Pirofski, Liise-anne 2014*). *I*t is used to describe an infectious agent like the following: virus, bacterium, prion, fungus, viroid, or parasite that causes disease in its host. The host may be a human, an animal, a plant, a fungus, or even another micro-organism (*Alberts B et al., 2002; MetaPathogen, 2015*). There are different types of pathogens which include the following: Bacterial, Viral, Fungal, Prionic Protozoa, Viroids and Human Parasite. Isolation It is defined as the complete separation from others of a person or plant suffering from contagious or infectious disease; quarantine. It is very damaging, and there is no way to ameliorate it and isolation of pathogen is the process whereby pathogens of yam are treated. This is the process where rotted yam tubers were rinsed in distilled water, surface sterilized with 70% ethanol and cut open with a sterilized knife and it is now treated.

Key words: Pathogens, treatment, isolation, identification, host, symptoms, prevention.

INTRODUCTION

Yam (*Dioscorea spp*) is a tuber crop belonging to the family Dioscoreaceae. The species of economic importance include *Dioscorea rotundata, Dioscorea alata, Dioscorea cayenensis, Dioscorea dumetorum, Dioscorea bulbifera and Dioscorea esculentus.* In West Africa, *Dioscorea rotundata* is also refered to as white yam or white guinea yam. It is the most widely cultivated. It is the most important food and income source for millions of producers, processors and consumers in West Africa. In 2012, world production of yam was estimated at 58.7 million tons with West Africa producing more than 92% (FAOSTAT, 2014). Nigeria and Ghana together produced about 66% of the world's yam supply. About 48 million tons of the tuber are produced annually in this sub-region on 4 million hectares of land. The five major yam producing countries are: Benin, Cote d'lvoire, Ghana, Nigeria and Togo. They account for 93% of world production. Nigeria alone accounts for 68% of global production (36 million tons on 3 million hectares). As food, yam plays an important role by providing cash and dietary carbohydrate to millions of people. The crop also makes a substantial contribution to protein in the diet, ranking as the third most important source of supply. It has a better keeping quality than most other tropical root and tuber crops because the tubers have an extended period of dormancy during which physiological activities are at a minimum. Yam can therefore, serve as an important food security crop. 31.8% of the population in Nigeria and 26.2 % in Ghana depend on yams for food and income security. Yam is also integral to the socio cultural life in this sub-region. *Dioscorea alata* has large starch grains, it is eaten boiled, roasted and as Ikokore (Ikpankwukwo and Ikokore) in some parts of Nigeria (Ijebu people in Ogun State).

A PATHOGEN

A pathogen or infectious agent is a biological agent that causes disease or illness to its host and it can infect unicellular organisms from all of the biological kingdoms. In the oldest and broadest sense, it is anything that can produce disease, a term which came into use in the 1880s (*"Pathogen". Dictionary.com Unabridged. Random House ; Casadevall, Arturo; Pirofski, Liise-anne 2014). I*t is used to describe an infectious agent like the following: virus, bacterium, prion, fungus, viroid, or parasite that causes disease in its host. The host may be a human, an animal, a plant, a fungus, or even another micro-organism (*Alberts B et al., 2002; MetaPathogen, 2015*). We have several substrates and pathways whereby pathogens can invade a host. The human body contains many natural defenses against some of common pathogens in the form of the human immune system and by some "helpful" bacteria present in the human body's normal flora. Also, we have some that have been found to be responsible for massive amounts of casualties and have had numerous effects on afflicted groups. Medical advances have been made to safeguard against infection by pathogens, through the use of vaccination, antibiotics and fungicide, pathogens continue to threaten human life and social advances such as food safety, hygiene, and water treatment have also reduced the threat from some pathogens (2016, Science Daily).

TYPES OF PATHOGENS

There are different types of pathogens and they include the following: Bacterial, Viral, Fungal, Prionic Protozoa, Viroids and Human Parasite.

Bacterial pathogens: Although the vast majority of bacteria are harmless or beneficial, a relatively small list of pathogenic bacteria can cause infectious diseases. One of the bacterial diseases with the highest disease burden is tuberculosis which is caused by the bacterium *Mycobacterium tuberculosis* and kills about 2 million people a year, mostly in sub-Saharan Africa. Pathogenic bacteria contribute to other globally important diseases, such as pneumonia, which can be caused by bacteria such as *Streptococcus* and *Pseudomonas*, and foodborne illnesses, which can be caused by bacteria such as *Shigella*, *Campylobacter*, and *Salmonella*. Pathogenic bacteria also cause infections such as tetanus, typhoid fever, diphtheria, syphilis, and leprosy. It can be killed by antibiotics because the cell wall on the outside is destroyed, expelling the DNA out of the body of the pathogen, therefore making the pathogen incapable of producing proteins and dies. It range between 1 and 5 micrometers in length. A class of bacteria without cell walls is mycoplasma. A class of bacteria which must live within other cells is chlamydia (genus), the world leader in causing sexually transmitted infection (STD).

Viral pathogens: This type of disease are the following smallpox, influenza, mumps, measles, chickenpox, ebola, and rubella. Pathogenic viruses are diseases mainly those of the families of: Adenoviridae, Picornaviridae, Herpesviridae, Hepadnaviridae, Flaviviridae, Retroviridae, Orthomyxoviridae, Paramyxoviridae, Papovaviridae, Polyomavirus, Rhabdoviridae, Togaviridae. Viruses typically range between 20-300 nanometers in length (Viral Special Pathogens Branch [26] Moved CDC).

Fungi pathogens: It comprise a eukaryotic kingdom of microbes that are usually saprophytes (consume dead organisms) but can cause diseases in humans, animals and plants. Fungi are the most common cause of diseases in crops and other plants. The typical fungal spore size is 1-40 micrometers in length.

Prion pathogens: Prions are infectious pathogens that do not contain nucleic acids. These abnormally folded proteins are found characteristically in some diseases such as scrapie, bovine spongiform encephalopathy (mad cow disease) and Creutzfeldt–Jakob disease (*The prion diseases* STANLEY B. PRUSINER, Scientific American).

Protozoa pathogens: These are single-celled organisms which can contaminate food and lead to infections such as amoebic dysentery with several symptoms like diarrhea. Some of these pathogens act as parasites and live on another organism (www.newhealthadvisor.com 2014, all rights reserved. Last updated 09 May, 2016).

Viroids pathogens: These are the smallest infectious pathogens known until now. They have very short strands of circular RNA – they don't have any protein coats though. These genomes can be extremely small and are usually between 246 and 467 nucleobases. They are generally plant pathogens, but some of them are human pathogens, especially the hepatitis D virus that's essentially a defective RNA virus.

Instead of coding for protein, viroids use RNA polymerase II for replication (www.newhealthadvisor.com 2014, all rights reserved. Last updated 09 May, 2016).

Human Parasite pathogens: Include a variety of worms and protozoa, which can cause several parasitic diseases in human. These parasites can be endoparasites, which causes infections inside our body. They can also be ectoparasites that infect us within the skin. The eggs and cysts of endoparasites are usually found in fece (www.newhealthadvisor.com 2014, all rights reserved. Last updated 09 May, 2016).

TREATMENT, HEALTH CARE AND PREVENTION

Bacteria can be treated with antibiotics. Viruses are treated with antiviral compounds. Infection with many pathogens are prevented by immunization and a small amount of pathogens are used in vaccines to make immunity stay alert and strengthen defense on the insides to prepare for a larger quantity of the virus ever getting inside. Hygiene is critical for the prevention of infection by pathogens.

How to prevent it are the following:

- **The Primary stage**: It involves public education about infectious diseases.
- **The Secondary stage:** It involves treating the actual infection that has already occurred by quarantining and/or vaccinating of infected individuals.
- **The Tertiary stage**: It also involves the recovery from illness.

ISOLATION

It is defined as the complete separation from others of a person or plant suffering from contagious or infectious disease; quarantine. It is very damaging, and there is no way to ameliorate it.

ISOLATION OF PATHOGENS

Rotted yam tubers were rinsed in distilled water, surface sterilized with 70% ethanol and cut open with a sterilized knife. About 20 pieces (3 mm in diameter) of the infected yam tissues were picked from the point of advancement of rot with a flamed sterilized forceps and inoculated on a solidified Potato Dextrose Agar (PDA) medium. Two replicates were made for each of the 10 yam tuber samples and the 20 plates inoculated on. The inoculated plates were incubated at room temperature (28°C) and observations made daily for possible fungal growth. Sub culturing was done to obtain pure cultures of the isolates. Stock cultures were prepared using slants of Potato Dextrose Agar (PDA) in McCarteny bottles and stored in a refrigerator at 4°C. Cultural characteristics of the fungi were observed and recorded Ezeibekwe, I.O. and Ibe A.E. (2010). Occurrence of the organisms was recorded as follows:

$$\frac{\text{Total number of figures}}{\text{Total number of fungal occurrence}} \times 100$$

IDENTIFICATION OF ORGANISMS

The identification of the isolates was done by examining the isolates macroscopically and microscopically. The colony characteristics, spores, mycelium either septate or not, conidium were taken note of. These structural features were matched with standards in Barnet and Barry (1972) and Booth (1971).

ISOLATION OF FUNGAL PATHOGEN

Small sections of yam tissues containing the advancing margin of rot and adjoining healthy tissue will be sterilized by immersion in 0.1% mercuric chloride solution for 1-2 minutes and rinsed three times in sterile distilled water. The peeled and sliced periderm of the rotted yams will then be planted on Potato dextrose agar (PDA) and incubated at 27± 2ºC for 7 days. The fungi that will grows will then be sub-cultured on Potato dextrose agar (PDA) and the rot fungi will be isolated and later identified .Fungi identification was carried out according to Domsch *et al.*,1980; Samson *et al.*,1984 and Rippon, 1958.

MICROBIAL ISOLATION

According to (Okigbo *et al.*, 2010) stated that yams will be peeled to expose the rot tissues by making use of a

sharp knife and forcept, an estimated 5 mm pieces will then be cut and surface sterilised by placing in a 10% sodium
hypochlorite solution for 2 minutes. The pieces will be removed, washed and rinsed by placing them in 3
changes of sterile distilled water for 3 minutes at each wash. They will be placed on sterile Whatman No. 1 filter
paper and put to dry in a laminar air flow cabinet for 5 minutes.

USES, PROPAGATION, GENERAL CARE, MAINTENANCE AND HARVESTING

Yams are used differently in different parts of the world. They are consumed after cooking by frying, boiling or roasting. The green parts of some plants can be cooked and consumed as a vegetable. Yams may also be used to produce flour or starch.

It is propagated vegetatively from small tubers and through vine cuttings. Land should be prepared for planting by plowing and harrowing. Tubers should be planted in trenches to a depth of 15 cm (6 in) allowing at least 30 cm (12 in) between individual plants and 1.5 m (5 ft) between rows. The soil is often mounded around plants or ridged to aid drainage. It is common practice to stake plants with a 2–4 m (6.6–13.2 ft) support to allow them to climb and ensure that all parts of the plant receive adequate sunlight. It requires 100 cm of water distributed evenly throughout the growing season. It should be mulched after planting to prevent plants from drying out. Failure to mulch the plants will result in drastic decreases in yield.

It can be harvested at any time after the leaves have started to yellow. The soil should be carefully dug around the tuber and the tuber cut from the vine. Harvesting is best carried out on sunny, dry days to prevent tuber rot.

YAM DRY ROT DISEASE

It is caused by yam Nematode *Scutellonema bradys* and poses a considerable phytosanitary risk because of its survival and ease of dissemination within yam tubers. Yams are propagated from whole tubers or pieces of tuber and are thus the principal means of dissemination of S. *bradys* in the yam growing areas of the world. Comparatively low populations of the nematodes in tubers do not produce external symptoms of damage (Bridge, 1973) and therefore the risk of dissemination by this means is greater. Infested seed tubers rather than soil are probably the main source of nematode inoculum in yam fields. The spread of the nematode in infested tubers has been highlighted between Islands in the Caribbean (Kermarrec *et al*., 1981, 1987).

HOSTS OR SPECIES AFFECTED

All yams specie *Dioscorea spp* grown for food are susceptible hosts of *S. bradys*. They are the following: *D. alata, D. cayenensis, D. dumentorum, D. esculenta* and *D. rotundata* (Baudin, 1956; Caveness, 1967; Smit, 1967; Bridge, 1982). Other yams known to be infected by *S. bradys* are *D. bulbifera, D. trifida* and *D. transversa* (Decker *et al*., 1967; Ayala and Acosta, 1971; Belliard and Kermarrec, 1978; Kermarrec *et al*., 1987).

Two wild *Dioscorea spp*. growing in forests in Nigeria and Cameroon have been shown to be natural hosts (Bridge, 1982; Bridge *et. al*., 1995).

SYMPTOMS

S. bradys causes a characteristic disease of yam (*Dioscorea spp*.) tubers known as 'dry rot disease'. Dry rot of yams occurs in the outer 1 to 2 cm of tubers directly associated with *S. bradys*. The initial stage of dry rot consists of cream and light-yellow lesions below the outer skin of the tuber. There are no external symptoms at this stage. As the disease progresses it spreads into the tuber, normally to a maximum depth of 2 cm but sometimes deeper. In these later stages of dry rot, infected tissues first become light brown and then turn dark brown to black. External cracks appear in the skin of the tubers and parts can flake off exposing patches of dark brown, dry rot tissues. The most severe symptoms of dry rot are seen in mature tubers especially during storage when it is often associated with general decay of tubers. No foliar symptoms have been observed on yams growing in soil infested with *S. bradys* (after Jatala and Bridge, 1990).

PREVENTION AND CONTROL

In Field Soil

By keeping fallow land free of all host plants is a suggested means of reducing damage by *S. bradys* to yams (Decker *et al*., 1967) but this is unlikely to be economic or practical in most situations. Yams are mostly intercropped with as many as other crops (Coursey, 1967). By controlling weed hosts and the exclusion of other crop hosts of *S. bradys* from around yams will help to reduce nematode damage. The population of soil of *S. bradys* will be reduced if a non-host or poor host crops, such as groundnut, chilli pepper, tobacco, Indian spinach (Beta vulgaris var. benghalensis), cotton, maize or sorghum are grown prior to yams (Adesiyan, 1976).

The application of chemical nematicides has, at best, only produced moderate yield increases and control of S. bradys (Anon., 1964; Ayala and Acosta, 1971) and information on the economics of this means of control is lacking for large scale use.

Resistance to S. bradys in yams has yet to be confirmed and all the main food yams *(D. alata, D. bulbifera, D. cayenensis, D. esculenta, D. rotundata*) are susceptible to damage. Resistance could prove to be the most practical and economic means of managing *S. bradys* if found in commercially acceptable cultivars.

By planting Clean planting material

Planting of nematode-free planting material is a practical and economic means of preventing damage by *S. bradys* and also their dissemination. Seed tubers showing symptoms of dry rot (cracking and flaking) should not be used for planting. The presence of dry rot in tubers without external symptoms can be determined by scraping away sections of tuber skin, or by the use of tuber pieces rather than whole tubers enabling the grower to examine for dry rot symptoms before planting. Bulbils or aerial tubers of the yam *D. bulbifera* and some forms of *D. alata*, which are used for propagation will be completely free of nematodes. A number of yams, such as *D. alata, D. rotundata* and *D. dumentorum*, can be produced from vine cuttings (Coursey, 1967). Even true seed can be used for propagating *D. rotundata* (Sadik and Okereke, 1975). Although these methods of propagation are not a practical means of producing ware tubers, they can be used to produce nematode-free seed tubers (Jatala and Bridge, 1990).

Also, the use of 'microsetts' or 'minisetts' cut from mature tubers (International Institute of Tropical Agriculture, 1984) can provide clean planting material if the mother seed yams selected are free of nematodes.

Traditional practices in Africa, the use of wood ash on yam tubers or mixing cow dung in yam mounds before planting are reported to decrease nematode numbers (Adesiyan and Adeniji, 1976).

Hot water treatment can also reduce or eliminate *S. bradys* from yam tubers. The expense of heating equipment, and the difficulties of maintaining constant temperatures, are the main prohibitive factors against its large scale use. However, it is feasible for small scale operations and for establishing nematode-free planting material. Most studies have shown that a water temperature of 50-55°C for up to 40 min gives the best control of *S. bradys* without damaging tubers (Jatala and Bridge, 1990).

References

[1] Adesiyan SO, 1976. Host range studies of the yam nematode, Scutellonema bradys. Nematropica, 6(2):60-63.

[2] Adesiyan SO, Adeniji MO, 1976. Studies on some aspects of yam nematode (Scutellonema bradys). Ghana Journal of Agricultural Science, 9(2):131-136.

[3] Alberts B; Johnson A; Lewis J; et al. (2002). "Introduction to Pathogens". Molecular Biology of the Cell (4th ed.). Garland Science. p. 1. Retrieved 26 April 2016.

[4] Anon., 1964. Nematode control trials with yam. Annual Report for the Ministry of Agriculture Nigeria for 1962-63.

[5] Ayala A, Acosta N, 1971. Observations on yam (Dioscorea alata) nematodes. Nematropica, 1:39-40.

[6] Coursey DG, 1967. Yams. London, UK: Longmans.

[7] Casadevall, Arturo; Pirofski, Liise-anne (11 December 2014). "Ditch the term pathogen". Comment. Nature (paper) **516** (7530): 165–6. doi:10.1038/516165a.

[8] Decker H, Casamayor GR, Bosch D, 1967. Observaciones sobre la presencia del nemátoda Scutellonema bradys en al tuberculo de ±ame en la provincia de Oriente (Cuba). CENTRO, Boletin de Ciencias Technologfa de la Universidad Central de Las Villas, 2:67-70.

[9] Domsch,R.H; Gan, W. and Anderson, I. (1980): *Compendium of Soil Fungi*. London Academic Press

[10] Ezeibekwe, I.O. and Ibe A.E. (2010). Fungl Organisms Associated with Yam (*Dioscorea rotundata*, Poir) Rot at Owerri, Imo State of Nigeria. Journal of Molecular Genetics. Year: 2010 | Volume: 2 | Issue: 1 | Page No.: 1-5.I and II pp 1-895.

[11] FAO 2014. Faostat.3.fao.ogr/faostat-gateway/go/to/download/Q/QC/E accessed on 01/08/2014.

[12] From Wikipedia, the free encyclopedia.

[13] International Institute of Tropical Agriculture, 1984. A system to increase seed yam production. Research Highlights for 1983. Ibadan, Nigeria: IITA, 103-107.

[14] Jatala P, Bridge J, 1990. Nematode parasites of root and tuber crops. In: Luc M, Sikora RA, Bridge J, eds. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Wallingford, UK: CAB International, 137-180.

[15] "MetaPathogen - about various types of pathogenic organisms". Retrieved 15 January 2015.

[16] Okigbo, R.N. and Emeka, A.N. (2010) Biological Control of Rot—Inducing Fungi of Water Yam (*Dioscorea alata*) with *Trichoderma harzianum*, *Pseudomonas syringae*, and *Pseudomonas chlororaphis*. *Journal of Stored Products and Postharvest Research*, **1**, 18-23.

[17] "Pathogen". Dictionary.com Unabridged. Random House.

[18] Rippon, J. N. (1988): The Pathogenic Fungi and Pathogenic Actinomycetes.In: *Medical Mycology*.3rd Edition Publ. Sanders Co. Philadelphia.pp163.

[19] Sadik S, Okereke OU, 1975. A new approach to improvement of yam, Dioscorea rotundata. Nature, 254:134-135.

[20] Samson, R.A; Hoekstra, E.S. and Van-Orschot A.N. (1984):*Introductory to Food Borne Fungi.*The Nertherlands Academy of Arts and Science. Pp11-25.

[21] *The prion diseases* STANLEY B. PRUSINER, Scientific American.

[22] Viral Special Pathogens Branch | [26] Moved | CDC.

[23] 2016, Science Daily.

[24] www.newhealthadvisor.com 2014, all rights reserved. Last updated 09 May, 2016.