Scientia Africana, Vol. 22 (No. 2), August, 2023. Pp 11-16
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

MORPHO-MOLECULAR IDENTIFICATION OF FUNGI ASSOCIATED WITH *FUFU*

Ikechi-Nwogu, C.G., *Odogwu, B.A. and Udongwo, N. I.
Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria. Email: chinyerum.nwogu@yahoo.com and *blessing.odogwu@uniport.edu.ng, elliotpikibo@gmail.com

Received: 14-04-2023
Accepted: 17-05-2023

https://dx.doi.org/10.4314/sa.v22i2.2

Journal Homepage: http://www.scientia-african.uniportjournal.info
Publisher: *Faculty of Science, University of Port Harcourt.*

ABSTRACT

Millions of people in Nigeria, Cameroon, Ghana, and other countries throughout the world eat fufu, which is produced from fermented cassava (Manihot esculenta). Despite being a one-stop shop for carbohydrates, it has been found to be a host for fungal pathogens. Based on morphology and the internal transcribed spacer (ITS) region, a study was done to isolate and identify the common fungal infections linked to fufu in storage. The most prevalent fungal isolate, FU-01, was examined under a microscope for the morphological investigation and was determined to be Geotrichum sp. Internal transcribed spacer (ITS) 1 and 4 (ITS-1F and 4R) regions of the isolate were sequenced and aligned with the sequence with accession number MG833313.1, which showed maximum similarity of 99% with Galactomyces candidum, also known as Geotrichum candidum sequences available at National Center for Biotechnology Information. This finding demonstrated that one of the fungi associated with preserved fufu is Geotrichum candidum, an environmental and acid-tolerant mold found in soil, water, air and as part of the human flora. This pathogen may have gotten into fufu as a result of contamination during pre-processing of cassava. It is strongly advised that fufu processors and handlers practice good personal hygiene and sanitation, heat the fufu before eating it, and closely monitor the production operations by the appropriate agencies.

INTRODUCTION

One of the major staple crop throughout the tropics and subtropics of the world is cassava (*Manihot esculenta*) (Filbert *et al.*, 2016). According to the reports of Obadina *et al.* (2007), the crop has the ability to help any country where it is cultivated economically, socially, and politically by acting as an import and export commodity and by giving its people a staple meal. The crop's stem, tubers, and leaves are all used. Cassava tubers, which are used to make *tapoica*, *fufu*, and *garri*, are the crop's most widely used by-products. Before being consumed, these cassava by-products go through a fermentation procedure to lower the amount of toxic cyanide they contain. In Nigeria, Ghana, and a small area of Cameroon, the by-product of fermented cassava known as *fufu* is consumed (Adebayo *et al.*, 2013). It is the most extensively consumed food in Nigeria and goes well with different kinds of soups and other dishes since it is readily available in ready-to-eat form. It is high in fiber, vitamin B and C, calcium, potassium, and carbohydrates (Inetianbor *et al.*, 2017; Uyoh *et al.*, 2009; Olopade *et al.*, 2014). Isirima *et al.* (2018) claims that fermentation

lowers the cyanide concentration by more than 60 percent. *Fufu* eating in excess can have harmful consequences on the body, especially if it wasn't adequately digested throughout the fermentation process. Some of these symptoms include cyanide poisoning, linamarin toxicity, and food allergies to mention a few. Usually, it is made by allowing peeled cassava root to ferment in water. In the fermentation process, lactic acid bacteria (*Lactobacillus plantarum*), yeast, and other bacteria play a crucial part in the breakdown of starch, acidity, detoxification, and flavor production. (Inietianbor *et al.*, 2017; Oyewole, 1991).

Due to the various methods involved in its preparation, and other human activities, *fufu* is vulnerable to fungi infection. Storage is essential since it allows for the extension of the period that *fufu* is available. *Fufu* unfortunately does not have a lot of storage alternatives. While having a shelf-life of up to seven days, some fungi-induced deterioration happens as a result of handling by processors, retailers, and customers. However, *fufu* deteriorates and spoils because of the fungi proliferation, which is influenced by processing, atmosphere, temperature, and moisture content. People of various racial and socioeconomic backgrounds use *fufu*. Therefore microbial growth, degradation, and microorganisms whose actions cause the growth of yeast and mold on *fufu* have all been linked to a parallel rise in food-borne illnesses and public health risks (Imade, 2021). Since the growth of these yeasts and molds causes the production of aflatoxins, accurate identification is essential. (Gnonlonfin *et al.*, 2013). To accurately identify these fungi pathogens, molecular characterization technique is vital in validating the morphological identification of species and strains for proper identification. This study therefore is aimed at isolating and identifying fungi species associated with stale *fufu* using morphological and molecular techniques.

MATERIALS AND METHODS

Source of sample

The *fufu* samples were bought from Choba market in Obio/Akpor Local Government Area of Rivers state, Nigeria in October, 2022.

Isolation of fungi from *fufu* using Blotter Method

The fungi associated with *fufu* were isolated at the Pathology/Mycology laboratory of the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria, using the traditional blotter method as modified by Ikechi-Nwogu *et al.* (2019)., for the investigation of the most common fungi isolate.

Morphological and microscopic characterization and identification of fungi associated with *fufu*

In order to identify the fungi, the isolate was incubated for seven days at room temperature in Potato Dextrose Agar medium. The isolate was morphologically identified and described by visually studying the mycelium using Snowdon's (1990) photographic reference. We compared the colonies' sizes, general colors, conidial colors, reverse colors, textures, zonation, and sporulation. The isolate was further examined microscopically using a light binocular microscope set to X40.

Molecular characterization using the Internal Transcribed Spacer (ITS) marker and identification of fungi associated with fufu

The manufacturer's instructions for Quick-DNATMFungal/Bacterial MiniPrepKit (Zymo Research Group, California, USA) were

Scientia Africana, Vol. 22 (No. 2), August, 2023. Pp 11-16
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

modified to extract the isolate FU-01's genomic DNA, which was then amplified at the Animal Science Molecular Laboratory at the University of Port Harcourt. The Nanodrop 2000c spectrophotometer was used to measure the quantity and concentration of the FU-01 isolate DNA (Thermo fisher Scientific Inc. Wilmington, Delaware, USA). The DNA purity was measured as a ratio of absorbance at 280 nanometer (nm) to that of 260 nanometer. The quality of the DNA of the isolates FU-01 was further quantified using the Agarose gel electrophoresis performed according to the modified method of Saghai-Maroof *et al.* (1984). The DNA sample of the FU-01 isolate was then shipped to the International Institute of Tropical Agriculture (IITA) Bioscience Center, Ibadan, Nigeria for amplification and sequencing. The primers used to amplify fragments of the nuclear ribosomal DNA (rDNA) of the FU-01 isolate were the Internal Transcribed Spacer 4 (ITS4) with the sequence TCCTCCGCTTATTG ATATGS and ITS5 with the sequence GGAAGTAAAAGTCGTAACAAGG. The amplicons were sequenced using the ABI 3500 capillary electrophoresis sequencer. The DNA sequence file was saved in the Bioedit file with extension.ab1. The sequence was analyzed using the Molecular Evolutionary Genetics Analysis (MEGA) version 7.0.26 software, and aligned using the Basic Local Alignment Search Tool for nucleotide (BLASTN) 2.8.0 version of the National Center for Biotechnology Information (NCBI) database

RESULTS

Morphological Identification of Fungi Associated with Fufu

The plated pure culture (Plate 1) and the morphological characterization of isolate FU-01 showed that the isolate FU-01 had white mycelium that was apparent to the naked eye. Additional characteristics like the organism's morphology/form, elevation, and surface revealed that the isolate FU- 01 belonged to the genus *Geotrichum sp*.

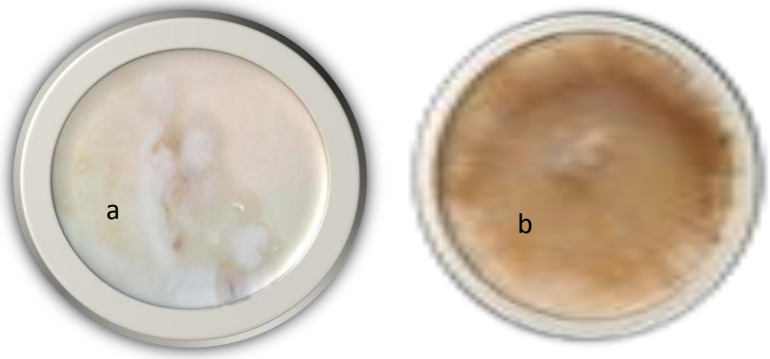


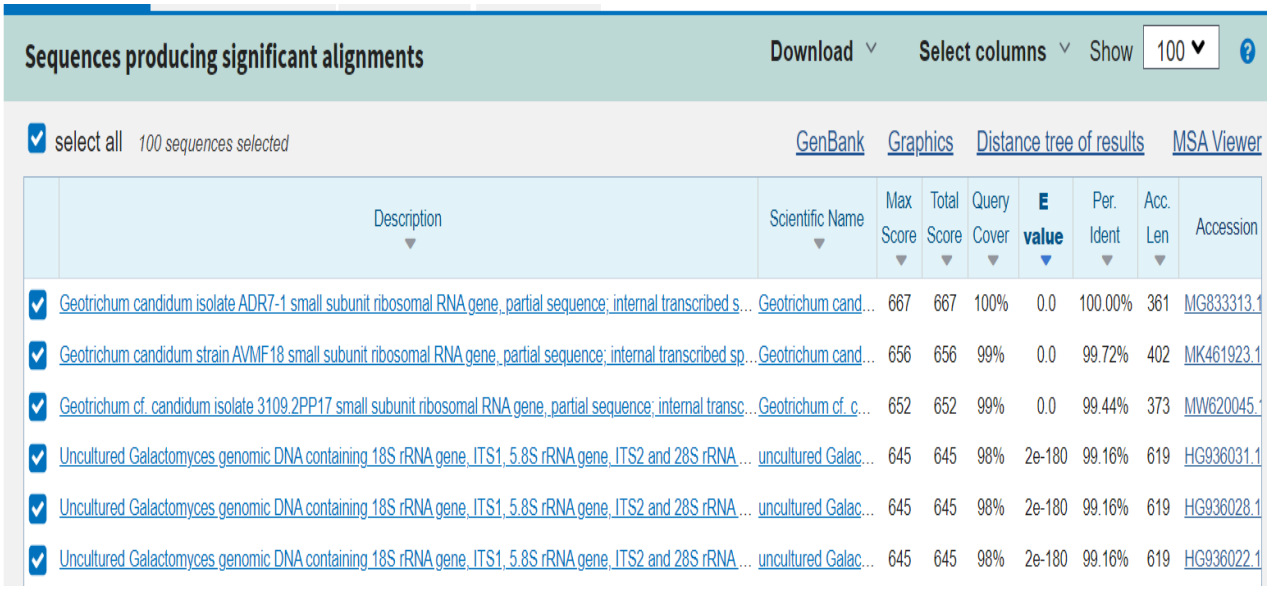
Plate 1: Pure culture of FU-01 fungi isolated from stored fufu

Table 1: Features of the FU-01 isolate

S/N	Descriptor	Feature
1	Form of mycelium	Circular with punctiform
2	Elevation of mycelium	Flat
3	Margin of the mycelium	Entire
4	Surface of mycelium	Creamy and powdery and
5	Conidia colour	White in colour

Molecular characterization FU-01 isolate

The result of the DNA sequence of the isolate FU-01 is presented in Fig. 1. From the result, the FU-01 isolate sequence aligned with 100 DNA sequences deposited in the composite biological database of National Centre Biotechnology Information (NCBI). However, the most identical sequence with similarity score of 100% and total score of 667, was the accession MG833313.1 identified as *Geotrichum candidum* formerly referred to as *Galactomyces candidum.*



Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Geotrichum candidum isolate ADR7-1 small subunit ribosomal RNA gene, partial sequence; internal transcribed s...	Geotrichum cand...	667	667	100%	0.0	100.00%	361	MG833313.1
Geotrichum candidum strain AVMF18 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp...	Geotrichum cand...	656	656	99%	0.0	99.72%	402	MK461923.1
Geotrichum cf. candidum isolate 3109.2PP17 small subunit ribosomal RNA gene, partial sequence; internal transc...	Geotrichum cf. c...	652	652	99%	0.0	99.44%	373	MW620045.1
Uncultured Galactomyces genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA ...	uncultured Galac...	645	645	98%	2e-180	99.16%	619	HG936031.1
Uncultured Galactomyces genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA ...	uncultured Galac...	645	645	98%	2e-180	99.16%	619	HG936028.1
Uncultured Galactomyces genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA ...	uncultured Galac...	645	645	98%	2e-180	99.16%	619	HG936022.1

DISCUSSION

This study was carried out to identify the fungi associated with stored *fufu* using morphological and molecular methods. Some common fungal species belonging to the genera *Aspergillus sp., Staphylococcus sp., Penicillium sp., Fusarium sp., Rhizopus sp. and Bacillus sp. or Escherichia sp*. have been reported globally to cause spoilage in *fufu* (Odom *et al.,* 2012; Inetianbor, 2017). However, from this study, the fungus *Geotrichum candidum* (known as the teleomorph *Galactomyces candidus*) was identified as the predominant fungal pathogen associated with the spoilage of *fufu*. The fungus belongs to the division Ascomycota, class Saccharomycetes, order Saccharomycetiales and family Dipodascaceae. Similar findings have been reported by Obadina *et al.* (2007) who claim that *fufu* is exposed to molds such *Aspergillus niger, Rhizopus spp., Geotrichum candidum, and Candida* spp. during storage and distribution. Initially, *Geotrichum candidum* was grouped as a yeast with moldy tendencies such as the baker's yeast Saccharomyces cerevisiae, but the genus **Geotrichum** and other related species have undergone extensive taxonomic revision (Ellis, 2023) and is **currently described as a mold (Wolfe, 2015).**

The fungi *Geotrichum candidum* is member of the human microbiome, it is associated with skin, sputum and faeces (Levetin et al., 2016). It is the causative agent of the human disease geotrichosis. *Geotrichum candidum* grows in the lumen of the bronchi. The disease is characterized as an endeobronchial infection (Levetin et al., 2016). Bronchial geotrichosis is similar to the allergic reaction of aspergillosis symptoms include prominent cough, gelatinous sputum, lack of fever and medium to coarse rashes (Patil, et al., 2014).

Scientia Africana, Vol. 22 (No. 2), August, 2023. Pp 11-16
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

The pathogen is also linked to the post-harvest storage rot of citrus fruits, tomatoes, carrots and other vegetables (Thornton *et al.*, 2010; Batt and Robinson, 2014). However, strains of the fungus is used in the production of certain dairy products such as cheese.

Improper fermentation contributes greatly in establishing mold in fufu. The presence of discoloration indicates that the *fufu* has been contaminated and as such not safe for human consumption. The odour from *fufu* discourages some people from consuming it. However, consumption of *fufu* infected with *Geotrichum candidum* definitely has a dangerous and negative impact on human health especially with those with weak immune system (Poirier et al., 2022). Many do not know that not all fungi can be eliminated by heating as those that are aflatoxin do not die after heat. Therefore, proper examination of fufu before consumption is important so as not to ingest already contaminated *fufu* and to be discarded appropriately.

CONCLUSION

From this study, the fungus *Geotrichum candidum* known in it telemorph form as *Galactomyces candidum* was identified as the fungal pathogen associated with spoilage of *fufu* based on morphological and molecular techniques. The presence of this pathogen in *fufu* could be as a result of pre-processing contamination. Good personal hygiene and sanitation from the handlers of *fufu*, heating of the *fufu* prior to consumption and close monitoring of the production processes by relevant agencies is highly recommended.

REFERENCES

Adebayo-Oyetoro, A.O., Eyewool, O. B., Obadina, A.O. and Omemu, M.A. (2013) Microbiological Safety Assessment of Fermented Cassava Flour ‘‘Lafun’’ Available in Ogun and Oyo States of Nigeria.Int. *J. Food Sci*., 1-5.

Batt, C. A., & Robinson, R. K. (2014). Encyclopedia of Food Microbiology Amsterdam. *Netherlands: Elsevier Science*, 195-250.

Gnonlonfin, G.J.B., Hell, K., Adjovi, Y., Fandohan, P., Koudande, D.O., Mensah, G.A., Sanni, A. and Brimer, L., (2013) A review on aflatoxin contamination and its implications in the developing world: a sub-Saharan African perspective. *Critical reviews in food science and nutrition*, *53*(4):349-365.

Imade, F., Ankwasa, E. M., Geng, H., Ullah, S., Ahmad, T., Wang, G. and Liu, Y. (2021) Updates on food and feed mycotoxin contamination and safety in Africa with special reference to Nigeria. *Mycology*, *12*(4), 245-260.

Inetianbor, J. E., Ehiowemwenguan, G., Yakubu, J. M. and Ogodo, A. C.(2014) In-vitro antibacterial activity of commonly used toothpastes in Nigeria against dental pathogens. *Journal of Advanced Scientific Research,* 5 (2): 40-45.

Isirima, C.B., Iyagba, A.G. and Oliseyenum, O.S. (2018) Organoleptic characteristics of cassava flour and fufu obtained from different cassava varieties (*Manihot* spp.) and tuber processing techniques. *Direct Research Journal of Agriculture and Food Science*, 6, 121–127.

Levetin, E., Horner, W.E., Scott, J.A., Barnes, C., Baxi, S., Chew, G.L., Grimes, C., Kennedy, K., Larenas-Linnemann, D., Miller, J.D. and Phipatanakul, W. (2016). Taxonomy of allergenic fungi. *The Journal of Allergy and Clinical Immunology: In Practice*, *4*(3), 375-385.

Obadina A., Olusola, O. and Odusami, A. O. (2009) Microbiological safety and quality

assessment of some fermented cassava products (*lafun, fufu, gari*), *Researchgate*, 27(2): 111-240

Obadina A.O. and Oyewole, O.B. (2006). Antifungal effect of L. plantarum on spoilage moulds in Ogi. *Research Journal of Biotechnology*, 1: 31-35

Obadina, A.O. (2007) Microbiological safety of cassava *fufu* processed in South West Nigeria.Proceedings of the 1st Federated African Nutrition Society (FANUS) Conference in Quarzazate.

Obadina, A.O. (2008) Baseline assessment of cassava *fufu* at different processing centres within South West Nigeria. Proceedings of the TWAS/BioVisionAlexandria. NXT for Young Scientists.

Odom, T. C., Udensi, E.A. and Nwanekezi, E.C. (2012) Microbiological qualities of hawked cassava *fufu* in Aba Metropolis of Abia State. *Nigerian Food Journal*, 30(1):53-58

Olopade, B. K., Oranusi, S., Ajala, R. and Olorunsola, S. J. (2014) Microbiological quality of fermented Cassava (*Garri*) sold in Ota Ogun State Nigeria. *International Journal of Current Microbiology and Applied Sciences*, 3 (3): 888-895.

Oyewole, O. B. (1991) Fermentation of cassava for *Lafun* production. *Food Laboratory News*, 17 (2): 29-31.

Patil, M., Jagdale, K., & Joshi, S. (2014). Pulmonary infection by *Geotrichum candidum*. *International Journal of Advances in Medicine*, 1(2): 171-172.

Poirier, M., Hugot, C., Spatz, M., Da Costa, G., Lapiere, A., Michaudel, C., Danne, C., Martin, V., Langella, P., Michel, M.L. and Sokol, H., (2022). Effects of Five Filamentous Fungi Used in Food Processes on In Vitro and In Vivo Gut Inflammation. *Journal of Fungi*, 8(9):893.

Uyoh, E., Ntui, V. and Udoma, N. (2009) Effect of local cassava fermentation methods on some physicochemical and sensory properties of fufu. *Pakistan Journal of Nutrition*, 8 (8): 1123-1125.

Wolfe, B. (2015). Geotrichum candidum: A yeast holding on to its moldy past. Retrieved from https://microbialfoods.org/geotrichum-candidum-mold-transition/ on the 13th of April, 2023