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GARRI AND AKAMU AS AN ALTERNATIVE MEDIA FOR THE GROWTH OF FUNGI

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ABSTRACT

The high cost of microbial culture media necessitates the screening and production of alternative media from cheap local materials. Mycological culture media has a great role to play in the growth and sporulation of fungi. In this study, the feasibility of using nutrient sources from Garri (a tuber product) and Akamu (a cereal product) to cultivate fungal species was investigated together with a conventional media-Sabouraud Dextrose agar (SDA) which served as control. Soil samples were serially diluted up to the 6th dilution and were inoculated into the SDA, Akamu and Garri medium using pour plate and spread plate techniques from the 4th dilution tube. Growth of the fungal species was observed to be slightly lower in the alternative media but was about the same to the control (SDA). The isolates were identified based on preliminary morphology and microscopic examination. They were species of *Aspergillus*, *Penicillium* and *Rhizopus*. From the percentage mean of each isolate, 89% of *Penicillium* grew on SDA. This was closely followed by Garri with 76% and Akamu with 32%. The frequency of isolation of *Aspergillus* was 38% on SDA, 37% on Garri and 65% on Akamu. *Rhizopus* recorded 64% in SDA, 57% in Garri and 16% in Akamu. SDA has been reported to be a better media for the cultivation of fungi but however, results from this study shows that hot-water made Garri and Akamu can compete favourably with SDA. Colony radical growth and sporulation of fungi were optimal for *Aspergillus* on Akamu (21mm) after 72hrs followed by *Penicillium* on SDA (18.2mm) after 72hrs The results from this research work shows that alternative media produced from maize and cassava (cheap raw materials) can be used for the cultivation of fungi in scenarios where acquiring conventional media is difficult.

KEYWORDS: Gari, Akamu, Fungi, Mould

INTRODUCTION

The increasing cost of microbial culture media has necessitated the continuous search for more readily available alternative culture media using local raw materials (potatoes, groundnut, cereals, cassava, etc…) at an affordable price. Different media for the growth and isolation of microorganisms have been reported from different substrates (Famurewa and David, 2008). Plant materials have been used to recover both fungi and bacteria from different sample sources such as maize, sorghum, groundnut, cassava, local food stuff waste etc.

Pelezar *et al*, (1993), defined a culture medium simply as any material in which microorganisms find nourishment and can reproduce themselves. Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. In the environment, microorganisms adapt to the habitats most suitable for their needs while in the laboratory, these requirements must be met by a culture medium (Simin, 2011). Microorganisms can obtain energy directly from sunlight while carbon can be made available in organic form such as carbohydrate.

The growth of microorganisms in an artificial medium is influenced by several physical and chemical factors. Microbiological studies depend on the ability to grow and maintain microorganisms under laboratory conditions by providing suitable culture media that offer favorable environmental condition including good carbon source, nitrogen source such as protein, enzymes vitamins, mineral elements such as phosphorus and sulphur, suitable pH, temperature, relative humidity, inorganic salt and water. For a microbiological media to fulfill its specific purpose, it must contain all the substances and compounds necessary for the growth and reproduction of the organism. Various substances have been combined into nutritive concotion and have successfully been used to isolate important microorganisms from materials such as water, soil, food, clinical specimens and dairy products (Okorondu *et al*., 2011). An optimal nutrient medium should provide not simply adequate growth, but the best possible growth (Meletiadis *et al*., 2001). The knowledge of the source of nutrients that encourage the growth of microorganisms is a useful determinant factor in considering the availability of the enzyme present in the microorganism which can be industrially useful.

Fungi are a group of eukaryotic spore-bearing, achlorophyllous organism that generally reproduce asexually and sexually. They are important in nutrient recycling department of nature (Khalid *et al*; 2006). Fungi due to their competitive saprophytic ability expressed by fast mycelial growth, spore production, presence of efficient and extensive system of powerful enzymes are able to utilize complex polysaccharides and protein as their carbon and nitrogen sources (Wubah, 1999). The aim of this research project is to investigate the feasibility of using garri and akamu as an inexpensive but effective alternative culture medium to conventional mycological media for isolating and identifying moulds.

MATERIALS AND METHODS

The materials required for this research work were collected from the Microbiology laboratory of the National Root Crop Research Institute, Umudike. Commercially prepared Akamu (Ogi) was brought from Isigate Umuahia Market together with Garri. The soil sample for the analysis was collected from the front of the microbiology laboratory of MOUAU.

3.2.1 Sterilization of Materials

The glasswares were washed carefully and packed into the autoclave for sterilization at 121^{o}C for 15 minutes (Chessbrough, 2002). Other materials were sterilized by rinsing them with ethanol.

Preparation of Media

The culture media used in this research work were Sabouraud Dextrose agar (SDA) which was used as a conventional medium for the growth of microorganisms. This served as the control for the experiment. The media was prepared by weighing appropriate quantity of the agar powder as stipulated by the manufacturers. The others used as test experiment were preparations of akamu and garri which were prepared with hot water. The garri was sieved to make the particles fine and smooth and prepared like Eba. 10g of Ogi was also dissolved in 30ml of distilled water before pouring of hot water to make the desired akamu gel.

3.2.3 Preparation of Inoculum Size

The soil samples were serially diluted by the 10 fold serial dilution technique. 10g (each) of the soil samples were dissolved in 100ml of distilled water from which serial dilutions were carried out by adding 9ml of distilled water into 10 different test tubes arranged in a test tube rack. Each of the tubes were labeled 10^{-1},10^{-2},10^{-3},10^{-4},10^{-5},10^{-6},10^{-7},10^{-8},10^{-9},10^{-10} respectively. 1ml of the dissolved soil samples was then collected using a sterile pipette and

added into the first test tube labeled 10^{-1} containing 9ml of distilled water. From the tube labeled 10^{-1}, 1ml of the soluble was taken and transferred to the second test tube labeled 10^{-2}. The same was done for all the test tubes. The dilution used for inoculation was the tube labeled 10^{-4}. Each of the test tubes was properly shaked before each transfer.

3.2.4 Inoculation of Plates

The pour plate technique (Dhawale and LaMaster, 2003) was used to inoculate the diluted soil samples into Petri dishes. An aliquot (IML) from dilution 10^{-4} was poured into a sterile Petri dish with a sterile 1ml pipette. Molten Sabouraud Dextrose Agar (SDA) and hot akamu (pap) were poured into the Petri dishes containing 1ml of the diluted soil samples. The plates were rotated gently for easy mix up of the diluted soil sample and media. The plates were allowed to solidify on the laboratory bench before incubating.

3.2.5 Incubation

The Sabouraud Dextrose Agar (SDA) plates were transferred into an incubator at 25^{o}C for 2-5 days. All the incubated plates were examined daily for the appearance of mycelial and colony growth.

3.2.6 Isolation and Identification

Macroscopic examination of the fungal isolates was done by observing the physical appearance and characteristics of the mycelia for colour and structure. A wet-mount preparation (Dhawale and LaMaster, 2003) of the isolates was done and then viewed under the x40 objective lens of the microscope. The morphological structures looked out for where the presence of septate, non-septate hyphae and the presence of sporangiophores.

3.2.7 Purification of Isolates

After incubation period, colonies of different shape and colours were observed on the plates. A pure culture of each colony type on each plate was obtained and maintained on agar slants. This was done by picking different coloured colonies and transferring them into small bottles containing sterile distilled water. Each of the suspension was subsequently sub-cultured onto SDA slants and incubated at room temperature (25^{o}c) for 4-5 days. The isolates were maintained and preserved in this form.

3.2.8 Lactophenol Cotton Blue Test

This test demonstrates the conidial arrangement or other reproductive structures or morphological forms which might give information toward the identification of the organism.

The lactophenol cotton blue wet mount preparation was used in observing the isolated fungi using the method of Dhawale and LaMaster, 2003. The preparation has three components: Phenol which kills any live organism, lactic acid which preserves fungal structures and cotton blue which stains the chitin in the fungal cell walls. A drop of LPCB stain was dropped on a clean glass slide. A needle was flamed over the burner, allowed to cool and dipped into sterile distilled water to wet the end of the needle. This was then used to pick a small portion of fungal spore and placed in the drop of LPCB stain. The mycelium was spread very well on the slide with the needle. A cover slip was gently placed over the slide. The slides were then mounted and examined with the X10 and X40 objective lenses.

RESULTS

Fungi are capable of utilizing various carbon containing compounds as carbon sources for growth. Conventional media-Sabouraud Dextrose agar (SDA), Garri (Eba) and Akamu (Pap) was successfully used for the cultivation and isolation of moulds from agricultural soil samples from various locations.

The occurrence and isolation of the various moulds that grew on each of the substrates used as culture medium in this research work were counted and is represented in table 1. The table shows the rate of occurrence of different fungal species on Garri, Akamu and Sabouraud Dextrose agar (SDA) at 10^{-4} dilution of the soil samples.

The pigmentation of each of the isolates on the culture plate was instrumental in their identification. Table 2 describes the microscopic and macroscopic features of the isolated fungal species. The cultural and morphological details of fungal growth on the respective nutrient sources were greatly enhanced. The most frequently encountered were species of *Penicillium*, *Rhizopus* and *Aspergillus*. The number of each of the isolates as they occurred in Garri, Akamu and SDA is presented in table 3. The mean colony diameter of each fungal isolate on the analysed samples is shown in table 4. Table 5 shows the percentage mean of each isolates on Akamu, Garri and SDA.

Table 1: Frequency/Occurrence of Various Fungal Isolates on Garri, Akamu and SDA.

Sample Analysed	Dilution used	Number of colonies		
		GA	SDA	AK
A	10^{-4}	18	22	10
B	10^{-4}	17	20	11
C	10^{-4}	17	18	10
D	10^{-4}	21	26	14
E	10^{-4}	20	21	9
F	10^{-4}	14	21	12
G	10^{-4}	18	17	13
H	10^{-4}	11	18	12
I	10^{-4}	15	12	9
j	10^{-4}	19	16	13

GA = Garri; SDA = Sabouraud Dextrose agar; AK = akamu.

TABLE 2: Colony Morphology and Microscopic description

Colour/colony morphology	Microscopic description	Microorganism
Black powdery spreading colonies	Well branched with colourless but septate, hyphae and black conidia. Conidiophores are upright, bearing phialides at the apex or radiating from the entire surface.	*Aspergillus* spp
Grey white	Sporangiophores are long, smooth-walled, simple or branched arising from stolons. They are filamentous and non-septate at the hyphae tip.	*Rhizopus* spp
Green	Conidiophores usually appear green or gray and are produced in chain on finger-like projection called phialides coming off the conidiophores.	*Penicillium* spp

Table 3: Number of Each Isolate on Garri, Akamu and SDA

Soil sample	GA			SDA			AKAMU		
	P	A	R	P	A	R	P	A	R
A	8	4	6	11	4	7	4	6	-
B	7	4	6	9	3	8	3	7	1
C	7	3	7	9	4	5	3	6	1
D	8	5	8	12	5	9	4	8	2
E	9	4	7	8	4	9	2	5	2
F	8	2	4	10	5	6	3	7	2
G	7	5	6	8	4	5	4	6	3
H	6	2	3	9	4	5	3	7	2
I	7	4	4	7	2	3	2	6	1
J	9	4	6	6	3	7	4	7	2

GA = Garri; SDA = Sabouraud Dextrose agar; P = *Penicillium*; A = *Aspergillus*; R = *Rhizopus*;

TABLE 4: Mean Colony Diameter (mm) Of Each Isolate on Garri, SDA and Akamu after 48 and 72 Hrs of Incubation.

Organism	48hrs (mm)			72hrs (mm)		
	SDA	GA	AK	SDA	GA	AK
Aspergillus	9	6	14	12.4	9.4	21
Penicillium	12	7.6	5.7	18.2	9.5	7.8
Rhizopus	8	5	3	10.1	8.3	5

Table5: % Mean of Each Isolate on Akamu, Garri and SDA

Soil samples examined	*Penicillium*			*Aspergillus*			*Rhizopus*		
	SDA	GA	AK	SDA	GA	AK	SDA	GA	AK
10	89%	76%	32%	38%	37%	65%	64%	57%	16%

DISCUSSION

Microbiological media provide an artificial means for the growth of microorganisms because they contain some essential nutrient that could enhance their growth and metabolism (Tharmila *et al*; 2011). These media are used for selective and differential cultivation of microorganisms. Due to the exorbinant cost of conventional media, screening of alternative medium in the form of garri and akamu was conducted. All fungi require a relatively large amount of carbon source and most utilize carbohydrate more readily than other carbon compounds (Hawksworth, 2001). Carbohydrate in the form of starch is abundantly present in Garri and Akamu. Thus starch-utilizing fungi would have enough of this nutrient in Garri and Akamu.

None of the carbon sources employed (in the form of Akamu, Garri and SDA) was inhibitory to the growth of fungi as they were used to successfully isolate moulds from the soil. However, the growth of fungi on each of them varied with some species of fungi growing more luxuriantly on a particular media type. The growths of these organisms were impressive as they were discrete colonies on each growth medium (Plates 1-111). The cultural and morphological details of fungal growth on garri were greatly enhanced but more pronounced in Akamu (Plate 11) and Sabouraud Dextrose agar (SDA).

From this study, the most dominant isolated fungi were *Penicillium* (89%) on SDA followed by *Aspergillus* (65%) in Akamu. *Penicillium* and *Rhizopus* showed their maximum growth on SDA but *Aspergillus* showed more radical growth (colony diameter; table 4) than any of the other medium. Akamu favoured the growth of *Aspergillus* sp (65%) more than any other species. Thus Akamu could act as a selective medium for cultivation of *Aspergillus* sp from

the soil. However, the association of *Aspergillus* with cereals calls for caution to cereal user (maize included) because of the toxin (Aflatoxin) associated with this species of fungi.

Table 1 shows the fungal counts on each of the medium used for growth and it indicates that SDA was the most readily utilized when compared with Garri and Akamu. However, both Garri and Akamu competed favourably with SDA as the difference between the counts varied only slightly. The radical growth (mycelia extension) was highest in Akamu for *Aspergillus* (21mm) followed by *Penicillium* (18.2mm) and least in Garri.

The growth of mycelia, spore formation and colony morphology of the isolated fungal species on Akamu and Garri were found to be similar as in SDA. For instance, *Aspergillus* produced rings of spores that radiated outward in both Akamu and SDA but was more obvious in Akamu (Plate 11). Several studies have also proved the possibility of using alternative local materials to replace conventional media. In a study by Adesemoye and Adedire; 2005, different fungal species were successfully cultured in alternative media using different cereals to replace potato in potato dextrose agar (PDA). Okorondu *et al*; 2011 also used Garri agar to isolate fungi species.

The result of this study revealed that Garri and Akamu are nutritious and has all it takes to support the growth of fungi; also the pH of Garri is a little acidic. This contributed to the growth of fungi and inhibited the growth of contaminant bacteria (Okorondu *et al*; 2011) that could grow on the more conventional media. Jain (2001) studied the effect of different culture media on fungal growth and found that SDA medium showed maximum growth and sporulation of all fungi studied. This was in agreement with the present research and findings except for *Aspergillus* that responded more to growth on Akamu. The results of this study are also consistent with the work of Tharmila *et al*; 2011 where local materials (Sago, Palmyrah tuber and Cassava tuber) were successfully used to grow fungi.

5.2 CONCLUSION

The relative performance of fungal growth on the alternative media, when compared with the conventional media illustrated a good growth. Therefore these alternative media can be used for the cultivation of fungi which is found to be cost effective in the face of the costly nature of getting conventional media.

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