



Original Research Article

# An attempt to formulate culture media for the culture of air-borne fungi using local plant flours

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This study was carried out to evaluate the possible use of local products as alternative and cost effective laboratory media used for the culturing of airborne fungi. Five food crop flours were used in this experiment with Malt Extract Agar [MEA] serving as a control. The poured plates were exposed to indoor air to isolate air-borne microbes. The cultures were incubated at temperature of 28°C±2 for a period of 120 hours where the total count of the fungi were recorded. Their growths on the formulated media were compared with the growth on conventional media. The fungi colonies recorded during the experiment were members of the genus *Aspergillus*, *Penicilium*, *Trichoderma*, *Rhizopus* and *Neurospora*. The total number of fungi colonies were higher in Cassava Flour Agar [CFA], Wheat Flour Agar [WFA] and Plantain Flour Agar [PFA] recorded than [MEA] the conventional mycological medium. Therefore, from the result of this experiment, there is hope for the use of local product culture media such as CFA, WFA, and PFA as suitable readily available and cost effective for *in-vitro* growth of the test organisms.

**Key words:** Culture media, airborne, fungi, plant flours, formulations.

## INTRODUCTION

Apart from gases, dust particles and water vapour, air also contains microorganisms such as bacteria, fungi, pollen, algae, yeasts and protozoans (Elston, 2007; Hargreaves et al., 2003). Since we all breathe in air, the microbial population in air is in constant interaction with human and animal life, both directly as source of pathogenic and beneficial microbes (Kellogg and Griffin, 2006) and indirectly through biological effects on atmospheric processes (Deguillaume et al., 2008). They can cause infectious diseases such as tuberculosis (Hauschild et al., 2010), Legionnaire's disease (Masalma et al., 2010), various forms of bacterial pneumonia, influenza (Bergeron et al., 2011), measles (Yuan et al., 2007), as well as some non-infectious diseases such as asthma, allergies (Hoppe et al., 2012; Heederik and Taylor et al., 2011). Among all of the aforementioned groups of microorganisms, bacteria and fungi are of great concern as the leading airborne pathogens that can lead to large economical as well as ecological consequences (McCartney et al., 2003). The

dispersal of microbes in air begins with the discharge of microbial cells or particles loaded with viable microbes (aerosol) to the atmosphere. It is followed by the subsequent transport via diffusion and dispersion of these particles and finally their deposition on any surface. This surface can be a new place/habitat or the same source. There are many factors within the physical environment that affect the launching, transport and deposition of bio aerosols. Particles that become suspended in the air column arise mainly from terrestrial and aquatic environments and are typically launched by air turbulence (Pepper 2011). Wind is the primary means of transport for bio aerosols. Bio aerosols can be deposited by a number of mechanisms, including gravity pulling them down, making contact with surfaces, or combining with rain which pulls the particles back down to the earth's surface (Pepper 2011). Environmental aero mycology constitute the major aspects of aerobiology mainly because of the dominance of fungal spores in the airspora (Tilak, 1991).

Fungi are very intriguing organisms to study, and one of their most interesting features is their diversity. Some are opportunists, whereas many are well characterized as potent pathogens. Pathogenic fungi have a broad spectrum of hosts, ranging from plants to humans. Fungal plant pathogens cause considerable losses in agricultural crops throughout the world. It is thought that over 10 000 different plant diseases are caused by fungi, which are not necessarily host-specific. Fungal airspora diversity and concentration vary with time of day, weather, season, geographical location and the presence of local spore sources (Gadekar, 2014). The least number of airborne spores was found to be in desert and the greatest in tropical and temperate regions (Lacey, 1981). Members of genera *Cladosporium* (up to 80%) and *Alternaria* (up to 60%) are the most abundant fungal entities with peak in late spring and summer, while penicillian (up to 70%) and *Aspergillus* (up to 40%) are more frequent in autumn and winter (Kosalec et al., 2005). It is estimated that 70 – 95 % of airborne viable fungi are of respirable size (< 4.7 $\mu$ m) (Li and Kuo, 1994). However, fungal fragments of sub-micron size can also be emitted from the microbial growth surface (Gorny et al., 2002; Kildeso et al., 2003). It has also been shown that these fragments may contain mycotoxins (Brasel et al., 2005).

Culture media are used for selective and differential cultivation of Microorganisms (Pelczar et al., 1993). Media for *in vitro* cultures can be classified as liquid or solid (Kuria et al., 2008). Based on the market value and the scarcity of culture media, screening of alternate media is found to be an important task (Tharmila et al., 2011). In the environment, microbes adapt to the habitats most suitable for their needs while in the laboratory, these requirements must be met by a culture medium (Simin, 2011). When a medium is being prepared for microbial growth, consideration must be given to the provision of carbon an energy sources and other growth factors that are essential for the organisms (Laleye et al., 2007).

Microorganisms can obtain energy directly from sunlight while carbon can be made available in organic forms such as carbohydrates or inorganic forms such as carbon dioxide (Madigan et al., 2000). Media used in the laboratory for the cultivation of microorganisms supply the nutrients required for cellular growth and maintenance. A wide variety of culture media is employed by the microbiologist for the isolation, growth, maintenance of pure cultures and identification of bacteria according to their biochemical and physiological properties (John, 2006). The main objective of this study was to determine the possible use of local products for the culture of air-borne fungi.

## MATERIALS AND METHODS

### Sterilization of Materials

Glass wares used for this research work were sterilized in an autoclave at 121°C for 30 minutes. Cork borer, sterile

rods, conical flasks, beakers, graduated cylinders, inoculating loops and petri dishes were wrapped in foil paper and were sterilized in the oven for 60 minutes at 160°C. Other materials that could not be autoclaved were surface sterilized with near absolute alcohol (95% ethanol).

### Conventional Media

Malt Extract Agar (MEA) was used for this study. The Malt Extract Agar (MEA) weighing 25g was dissolved in 500ml of distilled water, and stirred to mix and autoclaved. The medium was allowed to cool to body warm temperature (just before solidifying) before pouring it into the petri dishes.

### Preparation of Formulated Media

Five formulated media - Cassava Flour Agar (CFA), Yam Flour Agar (YFA), Wheat Flour Agar (WFA), Plantain Flour Agar (PFA) and Beans Flour Agar (BFA) were produced for this experiment (Figure 1-5). The cassava Dextrose flour weighing 25g was dissolved in 500ml of distilled water. Solution was sieved and autoclaved for 45minutes. Allow to cool and sieve again. 10g each of glucose and 2g of agar was added as nutrient supplements and gelling agent respectively, stirred to mix and autoclaved at 121°C for 15 minutes. Four other formulated media were also prepared using the above procedure.

### Airborne Fungi Culturing

All five formulated flour media including two conventional media; MEA and NA were poured and allowed to cool overnight. All poured plates in triplicates given the total of 21 plates were exposed to air in myxomycetology laboratory university of Benin for 5minutes to trap airspora. All exposed plates were covered, sealed and incubated at room temperature (29°C±2). Daily observations were made on all plates. Total fungal and bacterial colony count in  $\mu$ m was taken every 24hours. The mean of the replicates was taken. The experiment was terminated after 120hours of inoculation when some plates were fully covered with microbial growth in diameter. The result summary was further analysed biostatistically using a one way ANOVA.

### Identification of Fungi

Fungi growth in all the media were identified to the genus level directly from colonies on CFA, YFA, WFA, PFA, BFA and MEA (Figure 6) media using well established techniques of macroscopic and microscopic examination and standard reference works for the identification of moulds using lactophenol blue stain. A portion of the obtained culture was placed and teased out into a clean glass slide upon a drop of lactophenol using a sterile inoculating needles and covered with clean cover slip. It was then viewed under the microscope using x4, x10 and x40 objectives. Many isolates were further identified to species level using appropriate



**Figure 1:** Cassava flour



**Figure 2:** Yam flour



**Figure 3 :** Wheat flour



**Figure 4:** Plantain flour



**Figure 5:** Beans flour



**Figure 6:** Total Count of fungal colonies on formulated media and Malt Extract Agar.

Key: CFA = cassava flour agar, YFA = Yam flour agar, WFA = Wheat Flour Agar, PFA = Plantain Flour Agar, BFA = Bean Flour Agar and MEA = Malt Extract Agar

monographs.

### Biostatistical Analysis

One way ANOVA was carried out to test the Null hypothesis that there was no significant difference between total count of airspora colonies in formulated media and total count in conventional media.

The results of the analysis revealed that the total number of fungal colony counts in conventional media was significantly lower (32.5) than that in the formulated flour media with CFA (39.2), WFA (30.7), PFA (33.8), YFA (22.1) and BFA (13.1) (Table 1).

This implies that the total count of air-borne fungi colonies on formulated media compared with the total count of air-borne microbial colonies in the conventional medium are significantly different.

### RESULTS

Results of the study revealed that all the formulated media supported the growth of air-borne fungi, though, at varying degrees. The growth of the test organisms on the formulated media implies that the flours which were used in formulating the media contained the required nutrients for fungal growth.

### DISCUSION

One of the major constraints facing the study of micro-organisms including airspora for maximum exploitation is finding cost effective and suitable laboratory media for *in vitro* culturing.

The diversity of bacterial species present in atmospheric air is wide, for example, gram-positive cocci from *Staphylococcus* species, *Micrococcus* species, *Bacillus* species, as well as gram-negative species: *Pseudomonas* species, *Moraxella* species are common (Gandara et al., 2006; Kim and Kim, 2007; Obbard and Fang, 2003). The concentrations of gram-positive bacteria are generally higher than those of gram-negative bacteria in indoor air (Fox and Rosario, 1994).

Environmental aeromycology constitute one of the major aspects of aerobiology mainly because of the dominance of fungal spores in airspora (Tilak, 1991). It has been proposed that bioaerosol inside homes are representative of the outdoor atmosphere, since airborne spores penetrate through doors and windows (Chew et al., 2003). The concentration of these microflora in indoor air also vary with a number of factors such as geographical location, human activities in agriculture and industries (Adhikari et al., 2004). A large proportion of airspora transported in atmospheric air are phytopathogens. Approximately over 10,000 species are fungal pathogens in plants (Agrios,

**Table 1.** Nutrient value of the five essential food flours per 100g portion

	<b>Nutrient Component</b>	<b>Wheat</b>	<b>Cassava</b>	<b>Yam</b>	<b>Plantain</b>	<b>Beans</b>
1	Water %	40	60	70	65	52
2	Energy kcal	1369	670	494	511	198
3	Protein (g)	12.6	1.4g	1.5g	1.3g	13.22
4	Fat (g)	1.54	0.28	0.17	0.37	0.91
5	Carbohydrates (g)	71	38	28	32	35.5
6	Fiber (g)	12.2	1.8	4.1	2.3	11.1
7	Sugar (g)	0.41	1.7	0.5	15	5.64
8	Calcium (g)	29	16	17	3	-
9	Iron (mg)	3.19	0.27	0.54	0.6	4.29
10	Magnesium (g)	126	21	21	37	91
11	Phosphorus (g)	288	27	55	34	267
12	Potassium (g)	363	271	816	499	-
13	Sodium(g)	2	14	9	4	-
14	Zinc (g)	2.65	0.34	0.24	0.14	2.21
15	Copper (g)	0.43	0.10	0.18	0.08	0.458
16	Manganese (g)	3.99	0.38	0.40	-	0.812
17	Selenium (g)	70.7	0.7	0.7	1.5	-
18	Vitamin C (g)	-	20.6	17.1	18.4	-
19	Thiamin (B1) (g)	0.30	0.90	0.11	0.05	0.345
20	Riboflavin (B2) (g)	0.12	0.05	0.03	0.05	0.094
21	Niacin (B3) (g)	5.46	0.85	0.55	0.69	-
22	Pantothenic acid (B5) (g)	0.95	0.11	0.31	0.26	-
23	Vitamin B6 (g)	0.3	0.09	0.29	0.30	0.17
24	Folate Total (B9) (g)	38	27	23	22	-
25	Vitamin A (g)	9	13	138	1127	-
26	VitaminE Alpha to Copherol (g)	1.01	0.19	0.39	0.14	-
27	Vitamin K1 (g)	1.9	1.9	2.6	0.7	-
28	Beta carotene (g)	5	8	83	457	-
29	Lutein + zeaxanthin (g)	220	0	0	30	-
30	Saturated fatty acids (g)	0.26	0.07	0.40	0.14	-
31	Monounsaturated fatty acids (g)	0.2	0.08	0.01	0.03	-
32	Polyunsaturated fatty acids (g)	0.63	0.05	0.08	0.07	-

**Source:** (USDA 2016.)

2005).

In developing countries like Nigeria, the exorbitant cost of ready-made laboratory media is a major constraint in laboratory diagnosis of human and plant diseases. Moreover, the increasing cost of culture media has necessitated continuous search for more readily available culture media at affordable prices. The feasibility of developing alternate media to Malt Extract Agar (MEA) using local cereal species as the basal media was studied by Adesemoye and Adedire (2005). Different media for the growth and isolation of organisms have been reported from different substrates. Plant materials have been used to recover both fungi and bacteria from different sample sources such as Groundnut, Sorghum extracts, local food, leaf yam, maize and pigeon pea (Famurewa and David, 2008).

Results of the study revealed that all the formulated media supported the growth of air-borne fungi, though, at varying degrees. This is in conformity with the findings of Adesemoye and Adedire (2005), Tharmilla and Thavaranjit (2011) and Ruth et al. (2012) who reported the use of

alternative culture media for growing fungi. The growth of the test organisms on the formulated media implies that the flours which were used in formulating the media contained the required nutrients for fungal growth (Table 1). This is in line with the findings of Akinyele and Adetuyi (2005) and Silva et al. (2005), which shows that starch based agricultural material supports the good growth of fungal and fungus-like organisms. Microbiological studies depend on the ability to grow and maintain microorganisms under laboratory conditions by providing suitable culture media that offer favourable conditions (Domsch and Anderson, 1980). The nutrients in the flours included protein, carbohydrate, and minerals. Protein constitutes a significant portion of microbial cells and thus is necessary for the growth of microorganisms (Prescot and Harley, 2002). The protein content of the formulated media must have ensured a good supply of nitrogen while the carbohydrate content served as carbon source both of which are essential for good fungal growth. The mineral content of the flours in the formulated media was probably useful for some aspects of the fungi's metabolism.

Although moisture (water) is required by all organisms for life processes require water for digestion of nutrients (Pelczar et al., 1993). Cassava Flour Agar (CFA) supports the highest total count of air-borne among other formulations and conventional media (MEA) in this study. According to Meletiadis et al., (2010), optimal nutrient medium should provide not simply adequate growth but the best possible growth in order to allow moulds growth without restriction and express all phenotypes.

The conventional bacteriological media- Nutrient Agar supported the highest total bacterial colony count followed by beans flour agar (BFA). CFA, YFA, WFA and PFA which are mainly starch based crops showed poor growth of bacteria. It clearly indicates that bacteria require more protein source than carbon for growth.

Total count of fungal colony on the formulated media, when compared with count in conventional media, illustrated that Cassava Flour Agar (CFA) could be a good growth media for laboratory culture of air-borne fungi (Figure 1). Hence the alternative media produced from food crop flours can be used for the culture of fungi, which is found to be cost effective in the present scenario of getting conventional media.

Biostatistical analysis was used to further explain the growth rate of the test organisms on formulations and conventional media.

## CONCLUSION

From the results of this study, food crop flours contain minerals and nutrients that can meet the nutritional requirements of air-borne fungi. Therefore, they can be utilized as alternative materials in the formulation of culture media for the *in vitro* growth of fungi for teaching and research purposes. An important advantage of the food crop flours used in formulating the various media is that it is readily available in Edo State, Nigeria (where the research was carried out). In solving the problem of the shortage of culture media for laboratory practical, the result of this research will go a long way in ameliorating this problem. Further investigation is recommended in the application of modern tools and methods in the study of fungal physiology as this will assist in manipulation of other readily available local products into suitable media to further check the exorbitant cost of conventional media.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper.

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