UTILIZATION OF INDIGENOUS FRUIT PEELS IN FORMULATION OF AFFORDABLE FUNGAL CULTURE MEDIA

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Abstract

Fruits peel is the outer covering of most succulent fruits can be utilized in compounding different microbiological media for routine culture of fungi in the laboratory. This study was aimed to formulate growth media for some economically important fungi by using fruits peel materials such as pineapple, orange and mango. Fungal isolates such as *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium chrysogenum* and *Candida* sp. were inoculated separately into the medium prepared in conical flasks by mixing fruits peel powder (4 g in 100 ml distilled water) and incubated at room temperature (25°C±1) for 5 days. This study showed that some economically important fungi can be successfully grown in some fruits peel media. *Aspergillus niger* growth was recorded on medium containing pineapple and orange peels but did not grow on mango peel medium. *Rhizopus stolonifer* growth was observed on medium produced from pineapple and orange peels but didn't grow on mango peels medium. *Candida albicans* was recorded on medium containing pineapple, orange except mango and *Penicillium chrysogenum* was recorded on medium containing pineapple, orange except mango.

Keywords: Fruit peel, fungi, isolates, media

Introduction

Agricultural waste materials support the growth of fungi. Modern efficient agricultural practices enable huge productions of fruits and vegetables throughout the world. Banana, pineapple, mango and pawpaw are among the most widely cultivated fruits. Waste emanating from aforementioned fruits includes peel, pulp and seeds that constitute about 40% of the total mass. Majority of these waste materials were often improperly disposed; hence constitute huge environmental risk (Essien, Akpan, & Essien, 2005). Fruit waste dumping sites provide necessary impetus for proliferation of vectors, pathogenic bacteria and yeast to thrive. A popular approach to mitigating poor handling of fruit wastes is by disposing in landfills or incineration. However, these methods may directly cause acute air pollution and produce leachates that can easily contaminate ground water and destroy aquatic lives (Ali *et al.*, 2014).

The fruit peel contains simple and complex sugars that are metabolized by microorganisms *(*Saheed, Jamal, Karim, Alam, & Muyibi, 2013) and have received much attention for their conversion to bioethanol, biogas and animal feed. The cultivation of microbial cells (bacteria and fungi) that converts fruit waste into value added products such as biomass that can serve as animal feed supplement is a unique approach. Fruit peels have been exploited for the production of many high value products but its potential as fungal growth medium has not been widely reported.

Microbiological studies depend on the ability to grow and maintain microorganisms under laboratory conditions by providing suitable culture media that offer favorable conditions (Domsch & Anderson; 1980*)*. The nutrients in the waste include protein, carbohydrate and minerals. Protein constitutes a significant portion of microbial cells and thus necessary for the growth of microorganisms. (Prescot & Harley; 2002). The protein content of the formulated media should have a good supply of nitrogen while the carbohydrate content served as additional carbon source both of which are essential for good fungi growth. The mineral content of the waste in the formulated media was probably useful for some aspect of the fungi's metabolism.

The cost of most commercially available microbial media is rising at a fast pace. To tackle this problem, some new microbiological media should be designed which are efficient as well as cost effective. This may be achieved by using agricultural wastes as raw material for microbial media. Utilization of agricultural waste as a substrate for fungal culture for the production of value-added product has been reported which include cellulase production by some fungi cultured on pineapple waste (Omojasola & Jilani, 2008). Sugarcane bagasse has also been reported as an energy source for the production of lipase by *Aspergillus fumigates* (Nagvi et al. 2013).

A growth medium is a liquid or gel designed to support the growth of microorganisms. The commercially available media are very costly. Routine practical requires large amounts of media on regular basis for streak plate pour plate and spread plate experiments. Availability of indigenous low-cost media rich in nutrients that compares favourably with commercial media is the need of the day. The search for alternative, cheap media for use in laboratory for routine microbiological experiments is on going. Recent has been focused on finding alternatives to gelling agents of media, agar in particular, and media, in general, because of its exorbitant price (Tharmila et al., 2011; Mateen *et al.*, 2012; Ravimannan et al., 2014).

Generally, fungi are grown on Potato Dextrose Agar (PDA), Sabouraud's Dextrose Agar (SDA), Rose Bengal Agar (RBA) or Corn Meal Agar (CMA) which are relatively exorbitant to procure. Basically, every fungus requires carbon, nitrogen and energy source to grow and survive. Fruit peels may meet these requirements and work as a fungal growth medium and can replace expensive media in the market. The aim of the current study was to formulate a cost effective and efficient medium for fungal cultures, that is, *Aspergillus niger, Rhizopus stolonifer, Candida albicans and Penicillium chrysogenum us*ing fruit peel wastes as raw materials.

Methodology

Collection and Preparation Of Samples

Fruit peels were obtained from freshly collected fruits, namely, pineapple, orange and mango bought at Sayedero market, Ilaro, Ogun State. The collected fruit were then thoroughly washed and peeled. Fruits peel were air dried at room temperature for 10 days to remove the moisture. The dried peels were then ground separately into fine particles by mechanical grinder and sieved with 1 mm sieve size to remove debris. The powder was stored in polythene bag at room temperature for this research and the experiment was conducted at the microbiology laboratory of The Federal Polytechnic Ilaro, Ogun state.

Inoculum Preparation

The suspension of 4days old cultures of fungi (*aspergillus niger, penicillium chrysogenum, candida albicans* and *rhizopus stolonifer*) were used to study the qualitative and quantitative growth analysis. They were prepared in saline solution (0.85% sodium chloride).

The fungal cultures were inoculated into 50 ml of saline aseptically and incubated at room temperature for 5 hours. Saline solution with lower concentration helps fungi in their metabolism and growth process. Similarly, saline solution helps to maintain and balance the osmotic pressure of growth media.

Preparation of Fruit Peel Media

About 4.0 grams of dried fruit peel powder were added into the 100 ml of distilled water and sterilized by autoclaving at 121°lbs/pressure for 15 minutes. After sterilization, the fruit peel waste broths were cooled and then 1ml of fungal inoculum was transferred into it.

Isolation

Sterile cotton wool was moistened by dripping them into ethanol to clean the working surface before bringing out the plates from the incubator. Sub culturing was carried to obtain pure cultures of the isolates .The pure isolates were prepared on agar slant .Agar slant for the fungi isolates were preserved in McCarthey bottles and stored in 4^0C.

Cultural Identification

The isolates were identified on the basis of their morphological and circular characteristics. The isolates were identified by first mounting each fungi mycelium on a glass slide then stained with lactophenol in cotton blue dye, covered with a covered slip and they were viewed under a compound microscope. The slides were viewed under x40 objective lens. The physical characteristics were compared to Bergey's manual for identification.

Maintenance of Fungal Isolates

After identifying the fungi, the fungal colonies were sub-cultured in freshly prepared SDA plates. For this, fungal isolates which were selected and observed under microscope were selected as the base culture for sub culturing as new colonies. Fungal isolates were pricked with the help of inoculating loop and the loop was streaked gently over the fresh media plates aseptically. Lastly, the media plates with the isolates were incubated at room temperature for 5 days.

Qualitative Analysis of Fungal Growth

The inoculums added were incubated at room temperature for 3days. The presence/absence of the fungal growth was visually observed.

Results

Different types of fungal colonies were isolated in SDA plates initially (as standard). In order to get proper information on fungal colonies and to isolate the better fungal colony, the well-grown fungal colonies were identified and sub-cultured on duplicate agar plates. The four (4) fungi sub-cultured on agar media have significant colonial characteristics which are mentioned in Table 1.

Table 1: Colonial characteristics of fungal isolates on SDA agar

MEDIA USED	COLONY MORPHOLOGY	FUNGAL ISOLATE
SDA	Velvety, black, creamy.	*Aspergillus niger*
SDA	Creamy white with a flat dry colony.	*Candida albicans*
SDA	Colonies grow rapidly, resemble cotton candy. Turned into blackish colony due to ageing.	*Rhizopus stolonifer*
SDA	Dry chains of spores from brus shaped forming blue to blue-green pigment.	*Penicillium chrysogenum*

Table 2: Qualitative growth analysis of fungi in fruit peels media

FRUIT PEELS MEDIA	*A. niger*	*R. stolonifera*	*C. albicans*	*P. chrysogenum*
Pineapple	+	+	+	+
Mango	+	–	–	–
Orange	+	+	+	+
Pineapple + Orange	+	+	+	+
Pineapple+ Mango	+	+	+	+
Mango + Orange	+	+	–	+

Note: + (growth), -(no growth)

Discussion

The effect of three different fruit peel wastes with their combinations viz., Pineapple, Mango, Orange; Pineapple with Orange, Pineapple with Mango and Mango with Orange on the qualitative growth of *Aspergillus niger, Penicillium chrysogenum, Candida sp* and *Rhizopus stolinifer* were studied and results were given in Table 2. It was observed that the *Aspergillus niger* growth was recorded in the medium which contained Pineapple, Mango; Pineapple with Orange, Pineapple with Mango and Mango with Orange peels. The *Aspergillus niger* growth was not recorded in the medium containing Orange peels. *Rhizopus stolonifer* growth was noticed in the medium containing Pineapple, Orange, Pineapple with Orange, Pineapple with Mango and Mango with Orange peels. The *Rhizopus stolonifer* growth was not recorded in the medium containing Mango. The *Candida albicans* growth was noticed in the medium containing Pineapple, Orange; Pineapple with Orange and Pineapple with Mango peels. The *Candida albicans* growth was not recorded in the medium containing Mango and Mango with Orange. The *Penicillium chrysogenum* was noticed in the medium containing Pineapple, Orange, Pineapple with Orange, Pineapple with Mango and Mango with Orange peels. The *Penicillium chrysogenum* was not noticed in the medium containing Mango peels.

Conclusion

This present study has revealed that the fruit peel waste materials contain minerals and nutrients that can meet the nutritional requirements of some important fungi. Thus, they can be utilized as alternative materials in the formulation of culture media for the in vitro cultivation of fungi for industrial and research purposes. An important advantage of the fruit peels used in formulating the various media is that it is readily available in Nigeria. Readily available fruits like pineapple, orange, and mango can be taken as the base for formulating cost effective and useful fungal media. This study has shown that fungi such as *Aspergillus niger, Rhizopus stolonifer, Penicillium chrysogenum and Candida albicans* can be grown in fruit peels. Thus, this study helps to mitigate the cost burden for producing fungal growth media. In addressing the problem of the shortage of affordable culture media for laboratory practical, the result of this research will go a long way in ameliorating this problem. Further research is still needed in the application of modern tools and methods in the study of fungal physiology as this will assist in manipulation of waste materials into useable forms.

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