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FORMULATION OF ENRICHED CASSSAVA PEEL AGAR FOR INVITRO CULTIVATION OF FUNGI

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Abstract

A substantial amount of waste is generated annually from food processing industry including crop residues like peels. Cassava is predominantly consumed in boiled form, but substantial quantities are used to extract cassava starch, called tapioca. This study is carried out to assess the use of cassava peel in the formulation of media for cultivating fungi. Cassava peel Agar (CPA) and Enriched Cassava Peel Agar (ECPA) were formulated and used to culture two test fungi isolated from yam rot and the soil. The fungi used were *Aspergellus niger* and *Penicillium spp*. The test organisms were aseptically transferred using ethanol sterilized cork borer of 0.8 cm to the formulated media and control medium. The morphological characteristics of the test organisms were similar in color with that of the control. The formulated media supported the growth of the test organisms at various degrees. Morphological characteristics of the test organisms on the formulated media was determined. The Cassava peel used in the formulation contains moisture of (0.15%), Crude protein (4.6%), fat content of (4.0%), Fibre content (12.44%) Ash content (10.0%) Carbohydrate content (68.81%). The pH range of PDA (5.3±0.3), CPA (6.2±0.2) with ECPA (6.4±0.4) being the highest. It is concluded that cassava peels contain the nutrient that can meet the nutritional requirement of fungi.

Keywords: Cassava, Invitro, Aspergillus, Penicillium, Enriched.

Introduction

Media used in the laboratory for the cultivation of microorganisms supply the nutrients required for microbial growth (Akpabio et'al., 2018). A wide variety of culture medium are employed for the isolation, growth and maintenance of pure culture. (Lindquist 2015). Different formulated media for the growth and isolation of organisms have been reported from different substrates (Barnett et'al., 2019). Some vegetables and fruits have been used to cultivate both fungi and bacteria such as Gooseberry, carrot, tomatoes cabbages pumpkin etc. A medium may be formulated as either permissive with the intent of allowing the growth of whatever organisms, restricted or selective with the intent of only selecting for growth of a particular subset of those organisms (Baker *et al.,* 2016). This may take the form of a nutritional requirement, for instance providing a particular component such as lactose as the only source of carbon for energy including a particular antibiotics or other substances in order to select only organisms which are resistant to those substances. This correlates to some degree with defined and undefined (complex) media which are made from natural product and containing an unknown combination of very many organic molecules, while defined media can be precisely tailored to select organisms with varying properties (Baker *et al.,* 2016).

Despite advances in media production, inefficiencies in microbial growth and fermentation persist due to suboptimal media formulations. General-purpose media often fail to discriminate between desired fermentative microorganisms and contaminants, while existing selective and differential media may not adequately support the growth of certain strains under industrial conditions. Furthermore, the lack of comparative studies on media performance limits the ability to identify optimal formulations for large-scale fermentation. This study seeks to bridge this gap by designing and evaluating media tailored to enhance microbial growth while mitigating contamination risks posed by species such as *Staphylococcus aureus*.

SAMPLE COLLECTION

The cassava peels were obtained from Biu LGA of Borno state where the cassava was grown. The peels were transported to The Science Laboratory Department of Federal Polytechnic Bauchi in a polytene bag.

SAMPLE PROCESSING

The sample was washed, and dried. The dried cassava peels were pulverized into powdered form. It was then sieved with sieve of known pore size and stored

SOLID MEDIA FORMULATION

Approximately 0.23g of agar and 2g of the formulated pulverized Cassava peel was weight and dissolved in 40mls of distilled water. The media was sterilized at 121°c for 15minutes and approximately 20mls was distributed into a sterile petri dish prior to inoculation. (Barnett *et'al*., 2019).

ENRICHMENT OF FORMULATED MEDIA

Cooked Meat broth was used to enrich the Cassava peel media. The meat sample was collected from Gwallameji market. After proper washing, the meat was cooked and 1ml of the meat extract was aseptically dispensed into a sterile petri dish using a sterile syringe, then 20mls of sterilized formulated medium was added. The mixture was swirled a little and then allowed to gel. (Arulanantham *et al.,* 2012).

Microbial Inoculation

A wire loop was used for the inoculation, the wire loop was passed through a flame to remove contaminants, and a portion of the test organism was cut from the perimeter portion of the colony. This region of the colony represents the youngest growth and so was used as a standardized inoculum. And it was transferred onto the center of each of the formulated media, Cassava peel Agar (CPA) and Enriched Cassava Peel Agar (ECPA) respectively taking care not to cause any further damage to the organisms. The plates were aerobically incubated for 3-5 days. The diameter of the organisms was measured using a meter rule after every two days for a period of nine (9 days), then the lag phase period, specific growth rate and mean generation time of the organisms on the formulated media and the enriched media were recorded. And for comparative analysis, a conventional mycological media (PDA) was prepared according to manufacturer's specification and used as control. (Barnett *et al.,* 2019).

Characterization and Identification of Fungal Culture

A small amount of aerial growth of each fungus was removed using needles and transferred to a drop of lacto-phenol blue on clean slide. A needle was used to tease out the hyphae and a cover slip was placed over the preparation taking care not to trap air bubbles in the lacto-phenol blue. The slide was examined under the microscope using x10 and x40 objectives. The morphology and cultural characteristics was useful in the identification of the fungi making reference to Barnett et al. (2019).

RESULTS

The physical and morphological characteristics of the test organisms on the Cassava peel agar and Enriched cassava peel agar (Table 1) can be comparable to that on the potato dextrose agar. There was no much difference in the morphological characteristics.

The proximate composition of the cassava peel was determined (Table 2)
The pH range of the formulated media and the control media is shown in (Table 3) the enriched cassava peel agar has the highest pH of 6.4 ± 0.4 The growth rate of the test organism on both the formulated media and control media is shown in (Table 4) with ECPA having the highest days of microbial growth which is between 1-11 days for both the test organism. However, there were significant differences in the number of colonies produced on the two formulated media (Table Table 5) enriched cassava peel Agar (ECPA) has the lowest mean colony count of 3.4 cfu/ml for *A. niger* and 3.2 cfu/ml for *Penicillium*. Cassava peel Agar has 6.5 cfu/ml for *A. niger* and 5.6 cfu/ml for *Penicillium.* From the result it shows that the control media has the highest Mean colony count of 7.8 and 7.3 cfu/ml which represents *A. niger* and *Penicillium species* respectively.

Table 1: Physical and Morphological Characteristics of the Test Fungi

Media	Test organism	Colour of Colony	Shape of Colony	Size of Colony
CPA	◆ *niger*	Black	Round	Big
	Penicillium	Greenish	Irregular	Big
ECPA	◆ *niger*	Black	Round	Small
	Penicillium	Greenish	Irregular	Small
PDA	*A. niger*	Black	Round	Big
	Penicillium	Greenish	Irregular	Big

Key: CPA Cassava peel Agar
PDA Potato Dextrose Agar
ECPA Enriched cassava Agar

Table 2:Proximate Analysis of Cassava Peel

Component	CPA (%)
Moisture	0.15
Crude protein	4.6
Fat content	4.0
Fiber content	12.44
Ash content	10.0
Carbohydrate content	68.81

Key: CPA- Cassava peel Agar
PDA- Potato Dextrose Agar
ECPA- Enriched Cassava Agar

Table 3: pH Range of Cassava Peel Agar, Enriched cassava peel and the control media (PDA)

Media.	pH
PDA	5.3±0.3
CPA	6.2±0.2
ECPA	6.4±0.4

Key: CPA- Cassava peel Agar
PDA- Potato Dextrose Agar
ECPA- Enriched cassava Agar

Table 4: Days of Microbial Growth

Media	Test organism	Days
CPA	*Penicillium*	1-10days
	A. niger	1-9days
ECPA	*Penicillium*	1-11days
	A. niger	1-11days
PDA	*Penicillium*	1-9days
	A. niger	1-9days

Key: CPA- Cassava peel Agar
PDA- Potato Dextrose Agar
ECPA- Enriched Cassava Agar

Table 5: Mean fungal growth count on the formulated media and the commercially sold media(PDA).

Medium.	Mean colony count (cfu/ml)	
	A. niger.	***Penecillium***
CPA.	6.5± 0.05.	5.6± 0.1
ECPA.	3.4± 0.1.	3.2± .02
PDA.	7.8± 0.1.	7.3± 0.6

DISCUSSION

The Result of the study revealed that both formulated media (Cassava peel agar and the Enriched cassava peel agar) supported the growth of all the test organisms. (*Aspergellus niger* and *Penicillium*). This was in accordance with the finding of Westsejin and Okafor (2012). The growth of these test organisms on the formulated media implies that cassava peel and the enriched cassava peel Agar contained the required nutrients for microbial growth. This is also in conformity with the findings of Ubalua (2018) who reported that

agricultural waste material supports the growth of Microorganisms. (Afolabi*et al.*,2012). In his work on formulated media suggested that the protein content serves as nitrogen, carbohydrates as carbon source and the mineral content of waste used in formulation was probably useful for some aspect of fungi metabolism. The pH of the commercially sold media PDA was (5.3± 0.3) which was lower compared to the ECPA (6.4± 04) and CPA (6.2± 0.2). The colony size on ECPA was smaller compared to that on PDA. Also it took longer days for the growth on ECPA (1-11days) compared to PDA (1-9days). These suggest that the lower pH of PDA give faster growth of the test organisms. The mean fungal growth of *A. niger* and *Penicilliumspp* on the PDA has the highest colony count followed by CPA and ECPA having the lowest colony count this could be as result of the high pH of ECPA or high level of protein content of the ECPA.

CONCLUSION

Based on the findings of this study, it is concluded that cassava Peel extract contains nutrients and minerals that can meet the nutritional requirement of fungi thus they can be utilized as alternative substrate in the formulation of culture media for the cultivation of fungi. When compared with conventional media it showed a good growth of the test organism. An important advantage of this agricultural waste used in formulating this media is that it is readily available.

RECOMMENDATION

At the end of this work, it is recommended that further studies should be done on the use of cassava peel for media formulation so as to improve its efficiency. These include:

i. The use of cassava peel slant as a medium for the preservation of fungi.
ii. Apart from the selected microbes used, other microorganisms should be grown to ascertain that the formulated media is suitable for their growth.

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