

Full Length Research Paper

Potentials of cellulosic wastes in media formulation

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Potential use of cellulosic wastes as carbon and energy sources in selective media formulations was investigated. Two agar media, Czapek-Dox and Sabouraud's agar, were modified by substituting their carbon sources with cellulose, sawdust and sugarcane pulps. Then, two fungi; *Aspergillus niger* ANL301 and *Penicillium chrysogenum* PCL501, newly isolated from wood-wastes, were transferred to the unmodified and modified media and their growth was monitored for 120 h. Growth of the organisms on modified media containing sawdust and sugarcane pulp compared favorably with that obtained for the unmodified equivalents. Modified Czapek-Dox agar containing 2% (w/v) sawdust (Wood agar) and sugarcane pulps (Cane agar) gave 78.9 – 93.3% of the maximum growth obtained on Sabouraud's agar. The modified Sabouraud's agar containing sawdust (Wood-Pep agar) and sugarcane pulps (Cane-Pep agar) yielded 84.4 – 100% of the maximum growth on Sabouraud's agar. Cellulose-containing media gave a lower level of growth (60.0 – 66.7%) of that obtained for the unmodified media.

Key words: Selective media, cellulosic materials, *Aspergillus niger*, *Penicillium chrysogenum*.

INTRODUCTION

Nutritional requirements for growth, maintenance and reproduction by microorganisms, vary widely. This forms the basis for differential and selective media employed in microbiological studies (Harrigan and McCance, 1966). One major requirement of every microbial media is a source of carbon and energy. Some heterotrophic microorganisms are capable of synthesizing all the amino acids, vitamins and other compounds essential for a living cell from simple materials such as inorganic nitrogen salts as long as they have a source of carbon and energy (Seeley and VanDenmark, 1981). Although the materials used in the preparation of culture media are generally in the form readily assimilated by the microorganisms, complex organic substances are used in selective and industrial media, particularly for biodegradation and bioremediation purposes (Kastner et al., 1994). Cellulosic biomass is biodegradable and serves as carbon and energy source to an assortment of soil organisms, which utilize plant cell-wall materials (Grant

and Long, 1981). The ability of such microorganisms to thrive on cellulosic materials was exploited in isolating cellulolytic organisms from wood-wastes (Nwodo-Chinedu et al., 2005). There is a growing interest on the production of industrial enzymes such as cellulases required for enzymatic hydrolysis and bioconversion of cellulosic biomass into valuable products (Wu and Lee, 1997; Solomon et al., 1999). This has necessitated an intensive search for viable cellulolytic organisms capable of producing efficient plant cell-wall degrading enzymes with desirable properties (Smiths et al., 1996). There is therefore the need to develop a cheap and effective selective media for isolating and cultivating such microorganisms. In this study, we compared the growth of *Aspergillus niger* ANL301 and *Penicillium chrysogenum* PCL501, newly isolated from wood-waste dump in Lagos, on Czapek-dox agar, Sabouraud's agar and the modified equivalents containing cellulose, sawdust and sugarcane pulps as sole carbon sources. We report our data showing that the modified media formulated with sawdust and sugarcane pulp as carbon sources supported the growth of the microfungi as efficiently as the commercial media which were unmodified.

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Table 1. Carbon/protein source and relative growth of cellulolytic ofungi on the different agar media.

Media	Carbon source (g L ⁻¹)	Protein source (g L ⁻¹)	Relative growth (%)	
			<i>A. niger</i>	<i>P. chrysogenum</i>
Czapek-Dox Agar (CDA)	Sucrose (30)	NaNO ₃ (2)	83.3	78.9
CDA-Cellulose	Cellulose (20)	NaNO ₃ (2)	61.1	58.9
Wood Agar	Sawdust (20)	NaNO ₃ (2)	88.9	87.8
Cane-Agar	Sugarcane Pulps (20)	NaNO ₃ (2)	87.8	93.3
Sabouraud's Agar (SA)	Glucose (40)	Peptone (10)	100.0	100.0
SA-Cellulose	Cellulose (20)	Peptone (10)	60.0	66.7
Wood-Pep Agar	Sawdust (20)	Peptone (10)	93.3	84.4
Cane-Pep Agar	Sugarcane Pulps (20)	Peptone (10)	100.0	94.4

(CDA-Cellulose, Wood-agar and Cane agar are modified Czapek-Dox agar containing cellulose, sawdust and sugarcane pulps, respectively. SA-Cellulose, Wood-Pep agar and Cane-Pep agar are modified Sabouraud's Glucose agar containing cellulose, sawdust and sugarcane pulps, respectively).

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical grade Crystalline cellulose were obtained from Merck, Darmstadt, Germany. Peptone water was obtained from Sigma – Aldrich, Spain. Agar No.2 was obtained from LABM, Lancashire, UK.

Cellulosic materials

Sawdust of Abora wood (*Mitragyna ciliata*) was collected from Okobaba Saw-mill, Ebute-Metta, Lagos, Nigeria. Mature sugarcane stem (*Saccharum officinarum*) was purchased from Oshodi market, Lagos, Nigeria. The fibrous pulp was crushed, soaked overnight and washed thoroughly in distilled water to remove the residual sugars. Samples were sun-dried, ground with Marlex exceller grinder (Mumbai, India) and passed through a sieve (about 0.5 mm pore size) to obtain the fine powder used for the study.

Media preparations

Czapek-dox agar (CDA) consists of (per liter of distilled water) sucrose 30.0 g, NaNO₃ 2.0 g, KH₂PO₄ 1.0 g, KCl 0.5 g, MgSO₄·7H₂O 0.5 g, FeSO₄·7H₂O 0.01 g and agar (No.2), 20.0 g. Sabouraud's agar (SA) consists of (per liter of distilled water), glucose 40.0 g, peptone water 10.0 g and agar (No.2) 20.0 g. The modified Czapek-dox agar (CDA-Cellulose, Wood-Agar and Cane-Agar) and modified Sabouraud's Agar (SA-Cellulose, Wood-Pep agar and Cane-Pep agar) differed in the carbon source as shown in Table 1. The media were sterilized by autoclaving at 121 °C for 15 min, cooled and distributed into sterile petri dishes, and allowed to gel.

Organisms and growth studies

Isolates of *Aspergillus niger* ANL301 and *Penicillium chrysogenum* PCL501 maintained at 4 °C on Potato dextrose agar (PDA) slants were sub-cultured on fresh sterile PDA plates and incubated for 3 – 5 days. This was used as inocula for the growth studies. Flamed, sterile cork borer was used to punch holes inclosing discs (about 10 mm diameter) of the pure fungus on PDA plates. One disc of the fungus was aseptically transferred and placed at the center of the sterile agar plates of each media. The cultures were incubated at

30 °C. Growth of the organisms was monitored by measuring the diameter of fungal spread for 120 h at 24 h intervals.

RESULTS

The relative growth of *A. niger* ANL301 and *P. chrysogenum* PCL501 on the different media is shown in Table 1. The growth of the microfungi on the modified media containing sawdust and sugarcane pulps was comparable to that obtained on the unmodified equivalents. A comparatively lower level of growth was obtained with cellulose-containing media. Maximum growth of *A. niger* ANL301 on Czapek-dox agar and modified Czapek-dox agar - CDA-Cellulose, Wood agar and Cane agar - was 83.3, 61.1, 88.9 and 87.8% (of the maximum obtained on Sabouraud's agar). *P. chrysogenum* PCL501 gave 78.9, 58.9, 87.8 and 93.3% of the growth on Sabouraud's agar, respectively, for Czapek-dox agar and the modified Sabouraud's agar containing cellulose [CDA-Cellulose], sawdust [Wood agar] and sugarcane pulps [Cane agar]. Figure 1 shows the growth curves of *A. niger* ANL301 and *P. chrysogenum* PCL501 on Czapek-dox agar and the modified Czapek-dox agar. The growth obtained for *A. niger* ANL301 on modified Sabouraud's agar was 60%, 93.3% and 100%, (of that on the unmodified media) for cellulose [SA-Cellulose], sawdust [Wood-Pep agar] and sugarcane pulps [Cane-Pep agar]. The growth of *P. chrysogenum* PCL501 on modified Sabouraud's agar was 66.7, 94.4 and 84.4%, (of that obtained on Sabouraud's agar) for media containing cellulose, sawdust and sugarcane pulps. The growth curves of *A. niger* ANL301 and *P. chrysogenum* PCL501 cultured on Sabouraud's agar and the modified Sabouraud's agar are shown in Figures 2a and b.

DISCUSSION

This experiment was designed to evaluate the ability of modified media in which the carbon sources were sawdust, sugarcane pulps and cellulose to support the

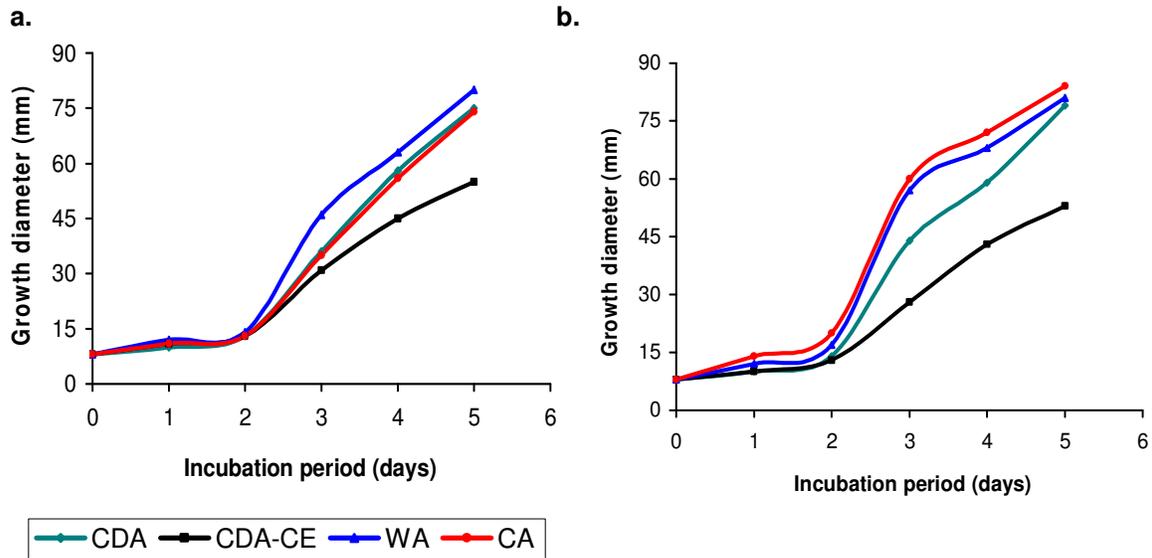


Figure 1. Growth curves of (a) *A. niger* and (b) *P. chrysogenum* on Czapek-dox and modified Czapek-dox agar. (CDA = Czapek-dox agar; CDA-Cellulose, WA [Wood agar] and CA [Cane agar] = Modified Czapek-Dox agar containing cellulose, sawdust and sugarcane pulps, respectively).

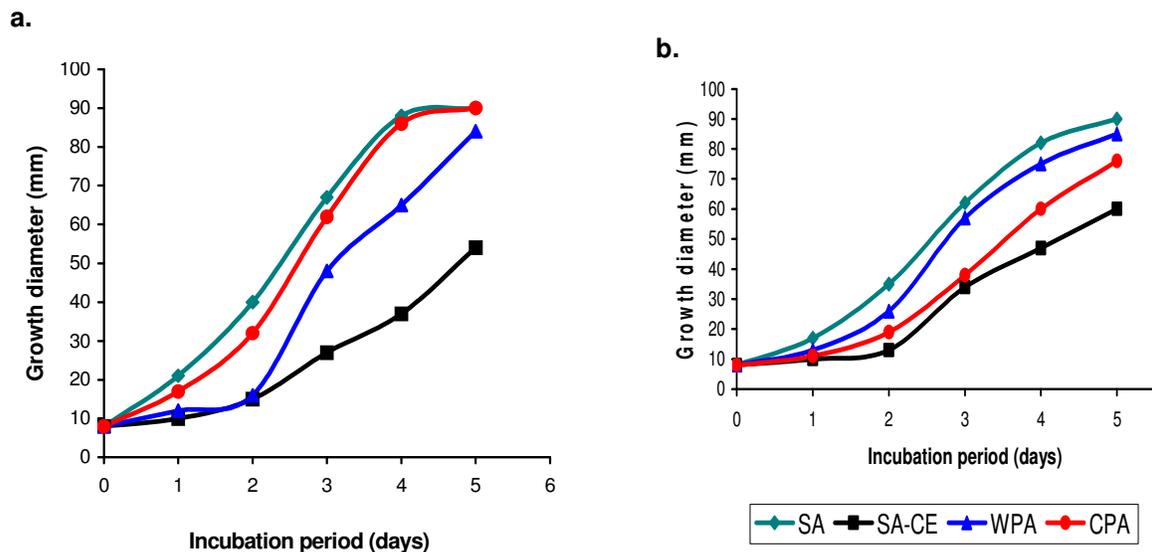


Figure 2. Growth of (a) *A. niger* and (b) *P. chrysogenum* on Sabouraud's Agar and Modified Sabouraud's agar. (SA = Sabouraud's agar, SA-CE, WPA [Wood-Pep agar] and CPA [Cane-Pep agar] = Modified Sabouraud's agar containing cellulose, sawdust and sugarcane pulps respectively).

growth of cellulolytic microfungi isolated from wood-wastes. Our results provide evidence that the growth of *A. niger* ANL301 and *P. chrysogenum* PCL501 on modified Czapek-dox agar containing sawdust (Wood-agar) and sugarcane pulps (Cane agar) was very close to that obtained on the unmodified media. A similar growth relationship was obtained with Sabouraud's agar and the modified equivalents containing sawdust (Wood-Pep agar) and Sugarcane pulps (Cane-Pep agar) respective-

ly. Cellulose-containing media gave a relatively lower growth compared to the unmodified equivalents. This could be attributed to adaptation of the organisms to cellulosic waste materials, since they were isolated from decomposing wood-wastes (Nwodo-Chinedu et al., 2005). Moreover, the organisms were found to produce extracellular proteins with high xylanase activity (Nwodo-Chinedu et al., 2006). Since, sawdust and sugarcane pulps contain other cell-wall polysaccharides such as xy-

lan in addition to cellulose, more simple sugars would be released in the media containing the complex cellulosic materials. This could account for the better growth of the organisms on the modified media containing sawdust and sugarcane pulps compared to that containing cellulose. Generally, the growth of the organisms on Sabouraud's agar (modified and unmodified) was better than that on Czapek-dox agar (modified and unmodified). This is understandable since Sabouraud's agar contains peptone (a mixture of amino acids and oligopeptides) which is an organic nitrogen source unlike Czapek-dox agar which contains inorganic nitrogen source that requires further metabolic processing into the organic form. A concentration of 2% (w/v) of cellulosic materials was found to be ideal for the growth of the microfungi. Although they could thrive at a lower concentration, the growth at 1% concentration and below was quite poor. At higher concentrations (3% and more), the media became very thick and jelly-like, thereby interfering with normal media preparation procedures. The media containing the cellulosic waste materials can be used to select, isolate and cultivate cellulytic microfungi. The use of these cellulosic wastes in media formulations would undoubtedly reduce the cost of microbial media. In addition, this would provide a means of transforming the vast quantities of waste cellulosic materials available in our environment into useful products and at the same time reduce the problem of waste disposal.

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