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Growth of Some Microorganisms on Media Formulated from Local Raw Materials

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Abstract: The growth of *Serratia marcescens*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Trichophyton interdigitales*, *Aspergillus niger* and *Mucor rouxii* was studied in 12 different types of media formulated from local raw materials. Their growth on the formulated media was compared with growth on conventional media (Nutrient Agar [NA] and Malt Extract Agar [MEA]). Maltose and sucrose were supplied as the carbon source, while growth factors were added. The media coded MCDF, MCjF and SCDF supported the growth of the bacteria studied luxuriantly when compared to nutrient agar (Biotec, UK). The fungi studied showed significantly better growth on the formulated media (on the basis of radial mycelia measurement) than Malt extract agar. The formulated media supported *Aspergilllus niger* and *Mucor rouxii* best among the test fungi. These media may be recommended as substitutes for the imported conventional media (NA and MEA) for the growth of these microorganisms locally, thereby conserving foreign exchange.

Key words: Culture media, Media from local raw materials, microbial growth on local materials

INTRODUCTION

Media are used for selective and differential cultivation of microorganisms (Seddon and Boriello, 1989; Taylor and Holland, 1989; Pelczar *et al*., 1993). When a medium is being prepared for microbial growth, consideration must be given to the provision of carbon and energy sources and other growth factors that are essential for the organisms. Microorganisms can obtain energy directly from sunlight (autotrophs) while carbon can be made available in organic forms such as carbohydrates or inorganic forms such as carbon dioxide (Madigan *et al*., 2000).

The increasing cost of culture media in Nigeria has necessitated continous search for more readily available culture media at affordable prices. Different media for the growth and isolation of organisms have been reported from different substrates. Laleye (1990) reported a modification of potato medium supplemented with cow dung, soy milk and other growth factors. These media supported the growth of the test organisms luxuriantly when compared with growth on conventional Nutrient (Oxoid) and Potato Dextrose Agar (Oxoid). Also Adesemoye and Adedire (2005) studied the feasibility of developing alternative media to Potato Dextrose Agar (PDA) using local cereal species as the basal media. They observed that all the fungal species grew to some extent better on the formulated media in relation to the standard set up.

This study therefore attempts to formulate media for the growth of microorganisms using local raw materials as growth factors with a view to making them available for use in laboratories, especially in developing countries.

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MATERIALS AND METHODS

Maltose and sucrose used for this study were obtained from BDH Chemical Ltd., Soybeans (*Glycine max*), African yam beans (*Sphenostylis stenocarpa*) and Pigeon pea (*Cajanus cajan*) were purchased from a local market in Ifon (Ondo State, Nigeria) while fresh cow dung was obtained from the town's abattoir.

The bacterial and fungal cultures used included *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Serratia marcescens*, *Aspergillus niger*, *Mucor rouxii*, *Trichophyton interdigitale* were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of Growth Factors

About 250 g of Soybeans, African yam beans, Pigeon pea were separately dehulled and blended in a Warring blender (Moulinex Mixer Blender 2, France) with occasional addition of water until liquor was obtained.

One hundred milliliter of each liquor was digested with 0.5 g trypsin (BDH, England) as described by Jassim *et al*. (1998). Also fish waste containing bones, fins etc. purchased from the market were sun dried and blended into a fine powder. Five grams of the power was dissolved in 100 mL distilled water and digested with trypsin as described above.

The husk from the leguminous crops and fresh cow dung were separately sun dried and blended into powder. Five grams of the powder was soaked in distilled water for 2-3 h, filtered through Whatman No. 1 filter paper and further centrifuged at 83 Hz for 10 min. The supernatant was used as the extract of each sample.

Preparation of Media

The compositions of the various media formulated are shown in Table 1. The media were decanted into test tubes sterilized in the autoclave at 121°C for 15 min.

Inoculation of Media

The various liquid and solid media were inoculated with 10^4 cfu mL^{-1} of the test organisms from a Nutrient broth (Oxoid) culture of 18-24 h. Visual assessment and comparison with growth on conventional media-Nutrient Agar (Oxoid) was carried out. Optical density measurement was done using Jenway 6505 (England) Spectrophotometer at 540 nm.

Fungal test organisms were inoculated with 3 mm mycelia plugs obtained from the growing edge of 72 h old cultures on Malt Extract Agar (MEA) (Merck, Darmstadt, Germany). The radial mycelia measurement was done as described by Aderiye *et al*. (1998).

Table 1: Composition of formulated media

Medium	Sugar* (%)	CD (mL)	OT (mL)	CJ (mL)	Fwd (mL)	Smd (mL)
CDF	1	10	-	-	10	-
OTF	1	-	10	-	10	-
CjF	1	-	-	10	10	-
CDS	1	10	-	-	-	10
OTS	1	-	10	-	-	10
CjS	1	-	-	10	-	10

*Sugar may be sucrose or maltose; CD: Cow dung extract, OT-African yam bean extract, Cj-Pigeon Pea extract, Fwd: Fish waste digest, Smd-soymilk digest, CDF: Cow dung extract fish waste digest medium, OTF: African yam bean extract fish waste digest medium, CjF: *Cajanus cajan* extract fish waste digest medium, CDS : Cow dung extract soymilk digest medium, OTS: African yam bean extract soymilk digest medium, CjS: *Cajanus cajan* extract soymilk digest medium

Microscopic Examination

Smears of the test organism were prepared from both the formulated media and those of the control (NA and MEA). The smears were observed under the microscope for physiological difference between the test organism which were grown on formulated media and the controls.

Statistical Analysis

Multiple comparisons among the means on the growth of the microorganism on the different media were made with the Duncan Multiple Range Test (DMRT) (Puri and Multen, 1980).

RESULT AND DISCUSSION

Basically, propagation of microorganisms in the laboratory, whether on a small or large scale, requires a nutrient environment (culture medium) which serves as a source of nutrient for multiplication (Baird *et al*., 1987). The ability of the twelve formulations to support the growth of the test organisms in comparison to growth on conventional media-NA (Oxoid) and MEA (Merck, Darmstardt., Germany) was investigated.

The growth of *Bacillus subtilis* and *Pseudomonas aeruginosa* was test supported by MCDF and MCjF (Table 2) with optical density (OD) reading of 1.40 and 1.00, respectively (Table 3). The exceptional growth rate on these media may be attributed to the versatility of *P. aeruginosa* and the presence of essential minerals provided in the growth factors. It has been reported that cow dung contains magnesium, calcium, iron, sulphur and di-potassium (Oyenuga, 1968) while digested fish waste has been shown to supply some essential amino acids to support growth, development and multiplication of organisms (Jassim *et al*., 1988; Oloke and Famurewa, 1991).

Generally, the results showed that media with sucrose as carbon source supported the growth of the bacterial test organisms better when compared with NA and formulations with maltose as carbon source. This, however, may be attributed to the availability of different pathways for the breakdown of glucose and fructose (monomers of sucrose) and it may be further supported by such phenomenon as obtained during diauxie growth. This is also corroborated by the findings of Oloke and Famurewa (1991). Lehninger (1994) has also shown that when a sugar made up of different monomers are exposed to acids or enzymes activity the simple monomers are released into the medium which are then utilized in order of physiological priority.

The formulated media suitably supported the growth of all fungi studied. However, the highest radial mycelia measurement obtained for *Aspergillus niger* and *Mucor rouxii* on the media supplemented with maltose as carbon source ranged from 19-45.5 mm when compared with growth on MEA (11-23.5 mm) (Table 4). This may be as a result of the preference of the organism for maltose as carbon source and the suitability of the nitrogen source provided in the formulations as growth factors. This assertion agrees with earlier reports of Olutiola *et al*. (1991) and Famurewa *et al*. (1994).

Table 2: Optical density reading for test organisms on maltose based media

Organisms	MCDF	MOTF	MCjF	MCDS	MOTS	MCjS	NA
Pseudomonas aeruginosa	1.40	0.80	0.77	0.54	0.50	0.46	0.90
Bacillus subtilis	0.90	0.75	1.00	0.44	0.49	0.47	0.85
Serratia marcescens	0.82	0.74	0.50	0.73	0.48	0.48	0.95

Values are means of triplicate readings

Table 3: Optical density reading on sucrose based media

Organisms	SCDF	SOTF	SCjF	SCDS	SOTS	SCjS	NA
Pseudomonas aeruginosa	1.20	0.42	0.41	0.72	0.84	0.41	0.85
Bacillus subtilis	0.93	0.45	0.45	0.83	0.47	0.51	0.80
Serratia marcescens	0.90	0.72	0.44	0.85	0.88	0.54	0.78

Values are means of triplicate readings

The relative performance of the bacteria organisms on the growth media formulated when compared with conventional media statistically showed that MCDF, MCjF and MOTS (Table 1) compared favourably with NA whereas when Sucrose was used (S) CDF performed better than NA and other formulated media (Table 6). MOTS (0.47) and MCD (0.57) also compared favourably with NA. The mean values obtained for CDF (1.04 and 1.01) were significantly different from all the formulated media when maltose and sucrose were used as carbon sources and also significantly from NA (0.81) when sucrose was used (Table 6).

The performance of the fungal organisms used in this study on the formulated media compare favorably with the conventional media (Tables 3-5 and 7). Their mean growth using both sugars as carbon source were not significantly different from the conventional media except for MCDS and MCjF (48 h) and MCDS, MOTS, MCjF (72 h) that compared favourably better (Table 7). The growth of *T. interdigitales* showed a general lag at the early stage of incubation on most of the formulated media. The radial mycelia measurement for MCDF was 8.5 and 39.5 mm after 24 and 72 h, respectively. Also on SCjF it was 10 and 39.3 mm after the same incubation period. This trend may be related to attempts by the organisms to adapt to the environment and most probably the organisms may be synthesizing the needed enzymes for the catabolism of the various nutrients. The enzymes are likely to be inductive (Stanier *et al*., 1985).

Table 4: Radial mycelial measurements (mm) (Maltose)

	MCDF		MOTF		MCjF		MCDS		MOTS		MCjS		MEA	
Organisms	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
A. niger	23.0	31.5	17.5	26.5	25.5	33.0	29.5	45.5	27.5	45.5	27.5	37.0	21.0	24.5
M. rouxii	27.5	32.5	25.5	29.5	29.0	34.5	35.5	36.5	24.0	25.5	14.0	23.5	14.0	15.0
T. interdigitales	21.5	39.5	24.5	57.0	29.0	44.5	34.5	52.5	28.5	47.5	26.0	42.0	28.0	45.0

Values are means of triplicate readings

Table 5: Radial mycelial measurements (mm) (Sucrose)

Organisms	Hours	SCDF	SOTF	SCjF	SCDS	SOTS	SCjS	NA
A. niger	24	19.5	28.5	28.7	18.0	14.5	34.0	21.0
	72	23.0	32.0	36.5	34.0	33.5	48.5	24.5
M. rouxii	24	24.0	28.0	21.5	21.0	31.5	18.0	14.0
	72	28.0	34.5	26.5	43.5	36.5	20.0	15.0
T. interdigitale	24	21.0	20.5	15.0	24.5	10.5	29.0	28.0
	72	41.5	42.5	39.5	39.0	14.0	46.0	45.0

Values are means of triplicate readings

Table 6: Growth of the bacterial species on the various media with different growth factors

	Media						
Growth factor	CDF	OTF	CjF	CDS	OTS	CjS	NA
Maltose	1.04^{a}	0.76^{bc}	0.76^{bc}	0.57^{cd}	0.49^{d}	0.47^{d}	0.90^{ab}
Sucrose	1.01^{a}	0.53^{c}	0.43^{c}	0.80^{b}	0.73^{b}	0.49^{c}	0.81^{b}

Mean values in each row with different superscripts are significantly different at p = 0.05

Table 7: Growth of the fungal species on the various media with different growth factors at different period

		Media						
Growth factor	Hours	CDF	OTF	CjF	CDS	OTS	CjS	NA
Maltose	48	24.40^{b}	22.33^{b}	27.83^{ab}	33.17^{a}	26.67^{b}	22.67^{b}	21.00^{c}
	72	34.50^{b}	37.67^{ab}	37.33^{ab}	44.67^{a}	38.50^{ab}	34.17^{b}	28.17^{c}
Sucrose	48	21.50^{a}	25.67^{a}	21.73^{a}	28.50^{a}	18.83^{a}	27.00^{a}	21.00^{a}
	72	30.83^{a}	36.33^{a}	34.17^{a}	31.33^{a}	28.63^{a}	28.63^{a}	31.67^{a}

Mean values in each row with different superscripts are significantly different at p = 0.05

Microscopic examination of the various test organisms after growth on the formulated media did not show any noticeable physiological changes when compared with those grown on conventional media. Hence, the formulated media can be recommended for use in place of conventional NA and MEA used in this study for culturing the organisms investigated.

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