International Journal of Biosciences (IJB)
ISSN: 2220-6655 (Print) 2222-5234 (Online)
Vol. 2, No. 5, p. 47-51, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Alternative vegetable nutrient source for microbial growth

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Received: 27 April 2012
Revised: 21 May 2012
Accepted: 22 May 2012

Key words: Culture media, media from local raw materials, conventional media, media formulation.

Abstract

The high cost of the microbial culture media paved a way for the production of alternative media using cheap local raw materials (Potato, Groundnut, Cereals, Cassava, Yam, Pigeon pea, Maize and Beans). The vegetables being a good source of nutrients are used to formulate both solid and liquid media for the growth of selected bacteria and fungi. Their growth on the formulated media was compared with growth on the conventional media. All the formulations produced good growth of microbes similar to the conventional media. In the solid media formulation, *Staphylococcus* sp., produced 230 CFU / ml in Formulation E, 150 CFU / ml of *Escherichia coli* in Formulation A, 170 CFU / ml of *Salmonella* sp., in Formulation B, 150 CFU / ml of *Klebsiella* sp., in Formulation B, 180 cells / 0.1 ml of *Aspergillus sp.,* in Formulation A and 150 cells / 0.1 ml of yeast in Formulation C. In broth formulations, *Staphylococcus* sp., *Escherichia coli*, *Klebsiella* sp., and *Salmonella* sp., produced 250, 200, 150, 175 cells / 0.1 ml of sample in the Formulations I, G, H and J respectively. Comparing with the performance on conventional bacteriological and mycological media, the prepared vegetable media is found to be a good and cheap media material for the isolation and cultivation of both bacteria and fungi.

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Introduction

Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. 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 and 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 increasing cost of culture media has necessitated continuous search for more readily available culture media at affordable prices (Hi Media Laboratories, Mumbai: Nutrient agar - ` 330/100 g; Sabouraud Dextrose agar - ` 399/100 g). 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 stuff waste, cassava whey, three – leaf yam, African oil bean, maize, beans and pigeon pea (Famurewa and David, 2008).

A modification of potato medium supplemented with cow dung, soy milk and other growth factors has been used for growth of the microorganism (Laleye, 1990). The feasibility of developing alternate media to Potato Dextrose Agar (PDA) using local cereal species as the basal media was studied by Adessemoye and Adedire (2005). With this background the present study was designed to formulate a media for the growth of microorganisms using local raw vegetables as growth factors (Carrot, Cabbage, Tomato and Pumpkin).

Materials and methods

Collection of samples

The vegetables like Tomato, Carrot, Cabbage and Pumpkin were purchased from Coimbatore Vegetable Market in a sterile plastic container. The collected samples were transported to the laboratory and processed immediately.

Formulation of media

The samples were washed thoroughly and five different formulations for both solid and liquid media were prepared.

Solid media formulation

The vegetables were cut manually into 3mm thickness. The sliced samples were subjected to dry heat in oven at 80 ^{o}C until the samples were completely dried. The dried samples were finely powdered using electronic blender and sieved into a fine powder. The powder was stored separately in sterile containers until its use (Umechuruba and Elenwo, 1999).

Five different solid media formulations (Formulation A to E) were prepared by mixing the samples in different ratios with 2 g agar, which is a solidifying agent and 0.001gm Sodium chloride in 100ml distilled water. The pH was maintained at 7 ± 0.5 for bacteria and 5 ± 0.5 for fungi (Table 1). The media was sterilized at 121 ^{o}C for 15 minutes and approximately 20 ml of the sterilized medium was distributed into each of the sterile petridishes.

Liquid media formulation

The fresh raw vegetables were crushed using mortar and pestle to extract the fluid. The extract was then filtered using suitable filters. Five different liquid

media formulations (Formulation F to J) were prepared by mixing the extracts of each samples in different ratios with 0.001gm Sodium chloride and made up to 100ml with distilled water. The pH was maintained at 7 ± 0.5. The media was sterilized at 121 ^{o}C for 15 min (Table 2).

Table 1. Formulation of solid media using vegetables (g/100ml).

Formulation	Vegetables (g)				Sodium chloride (g)	Agar (g)
	Carrot	Tomato	Cabbage	Pumpkin		
A	0.3	0.25	0.25	0	0.001	2
B	0.15	0.25	0.25	0	0.001	2
C	0.15	0.25	0	0.15	0.001	2
D	0	0.5	0	0.3	0.001	2
E	0.3	0	0.5	0	0.001	2

Table 2. Formulation of liquid media using vegetables (ml/100ml).

Formulation	Vegetables (ml)				Sodium chloride (g)
	Carrot	Tomato	Cabbage	Pumpkin	
F	0.3	0.25	0.25	0	0.001
G	0.15	0.25	0.25	0.15	0.001
H	0.15	0.25	0	0.15	0.001
I	0	0.5	0	0.3	0.001
J	0.3	0	0.5	0	0.001

Table 3. Nutritional status of vegetables.

Nutrients	Vegetables			
	Cabbage	Carrot	Tomato	Pumpkin
Protein (g)	0.95 ± 0.01	0.59 ± 0.01	1.08 ± 0.01	1.76 ± 0.04
Carbohydrate (g)	4 ± 0.15	8 ± 0.08	3 ± 0.15	2.1 ± 0.15
Potassium (mg)	147± 0.31	183 ± 0.58	292 ± 0.35	564 ± 0.36
Phosphorous (mg)	25 ± 0.58	23 ± 0.55	30 ± 0.30	74 ± 0.20
Magnesium (mg)	11 ± 0.20	8 ± 0.20	14 ± 0.30	22 ± 0.21
Calcium (mg)	36 ± 0.50	23 ± 0.50	12 ± 0.25	37 ± 0.21
Iron (mg)	0.13 ± 0.01	0.27 ± 0.01	0.33 ± 0.04	1.4 ± 0.15
Sodium (mg)	6 ± 0.10	5 ± 0.23	6 ± 0.15	2 ± 0.12
Zinc (mg)	0.15 ± 0.01	0.3 ± 0.03	0.21 ± 0.02	0.56 ± 0.02
Copper (mg)	0.013 ± 0.00	0.052 ± 0.00	0.073 ± 0.00	0.223 ± 0.00
Manganese (mg)	0.154 ± 0.00	0.062 ± 0.00	0.14 ± 0.01	0.218 ± 0.00
Selenium (mcg)	0.5 ± 0.05	0.2 ± 0.02	0.2 ± 0.01	0.5 ± 0.02

Values are mean ± SD of triplicates

Microbial inoculation

Solid media

The standardized culture (0.1 ml of overnight culture) of each test bacteria (*Staphylococcus* sp., *Escherichia coli*, *Salmonella* sp., and *Klebsiella* sp.,) and fungi (*Aspergillus* sp., and Yeast) were inoculated into the media of different formulations (A to E) by spreading. Organisms introduced on Nutrient agar and

Sabouraud Dextrose agar media serve as control. The inoculated plates were incubated at 37 ºC for 24 - 48 h for bacterial growth and at room temperature for 3-5 days for fungal growth. After incubation the plates were observed for bacterial and fungal colonies and the degree of growth was compared to the conventional media (Nutrient agar and Sabouraud Dextrose agar).

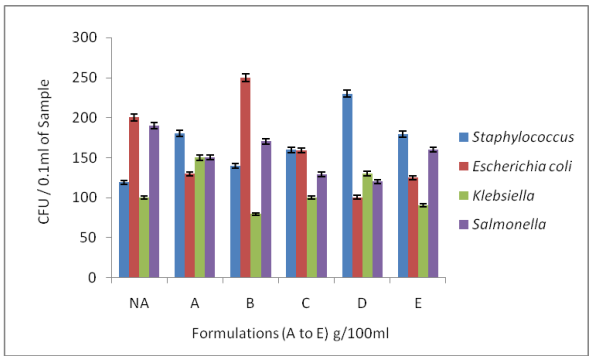
Liquid media

A loopful of the standard overnight culture of each test bacteria (*Staphylococcus* sp., *Escherichia coli*, *Salmonella* sp., and *Klebsiella* sp.,) was subjected to inoculation in the different formulated broths. The bacterial control was maintained in Nutrient broth. The inoculated flasks were incubated in orbital shaker at 120 rpm for 24 h at 37 ºC for bacterial growth. After incubation the degree of growth was measured using Petroff – Hausser and compared with the growth in Nutrient broth.

Results and discussion

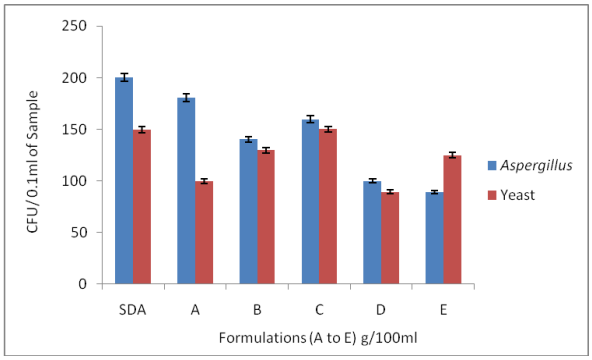
Media are used for selective and differential cultivation of Microorganisms (Seddon and Boriello, 1989; Pelczar *et al*., 1993). Media for *in vitro* cultures can be classified as liquid or solid (Kuria et al., 2008). Based on the market value and the scarcity of culture media, screening of alternate media is found to be an important task (Tharmila *et al*., 2011). Hence an alternative media using fresh raw vegetables (Carrot, Cabbage, Tomato and Pumpkin), which supply the needed vitamins and minerals, was designed and the ability of the five different formulations of both solid and liquid media to support the growth of the test organisms in comparison to the growth on conventional media was investigated.

The formulated media supported the growth of all test organisms by serving the nutrients essential for its growth (Table 3). In the solid media formulation, *Staphylococcus* sp., *Escherichia coli* and *Klebsiella* sp., produced 230, 250 and 150 CFU / 0.1 ml of the sample in the Formulation D, B and A respectively, which is higher than 120, 200 and 100 CFU / 0.1 ml of the sample in Nutrient agar media. *Salmonella* sp., showed a growth range of 170 and 190 CFU / 0.1 ml of the sample in Nutrient agar and Formulation B. *Aspergillus* sp., and Yeast resulted in 180 and 150 CFU / 0.1 ml of the sample in Formulation A and C, where the Sabouraud Dextrose agar showed 200 and 150 CFU / 0.1 ml of the sample. With respect to the formulated broths, *Staphylococcus* sp., produced equal number of colonies in both control and formulation I (250 cells / 0.1 ml of the sample). The formulation G & H showed a better of *Escherichia coli* and *Klebsiella* sp., (200 and 150 cells / 0.1 ml of the sample) when compared to the conventional broth (190 and 90 cells / 0.1 ml of the sample). *Salmonella* sp., generated 200 and 175 cells / 0.1 ml of the sample in the Conventional broth and Formulation J.



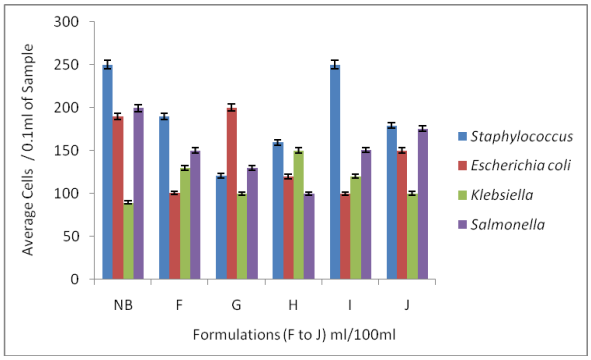
*Vertical bars indicates the SE of triplicates

Fig. 1. Growth of bacteria in different solid formulations.



*Vertical bars indicates the SE of triplicates

Fig. 2. Growth of fungi in different solid formulations.



*Vertical bars indicates the SE of triplicates

Fig. 3. Growth of bacteria in different liquid formulations.

The relative performance of the bacterial and fungal growth on the formulated media, when compared with the conventional media illustrated a good growth of *Staphylococcus sp*., *Escherichia coli*, *Klebsiella sp., Salmonella sp.,* and fungi (Fig. 1, Fig.2 & Fig.3). Hence the alternative media produced from raw vegetables can be used for the cultivation of microbes, which is found to be cost effective in the present scenario of getting conventional Medias.

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