BACK TO KITCHEN: FOOD-GRADE MEDIA IS A LOW-COST ALTERNATIVE TO COMMERCIAL BACTERIOLOGICAL MEDIA

IMMANUEL REBECCA, PATIL ROHINI PRADEEP

Abstract: The high cost of the microbial culture media paved a way for the production of alternative media. Foods are not only of nutritional value to those who consume them but often are ideal culture media for microbial growth. The study aimed to find suitable substitute of agar and also to prepare culture media from local food substrates like carrot, tomato, spinach and soya bean as nutrient supplements to culture routinely used microbial cultures. An additional advantage of these nutrient sources is that, they are available all year round at a fairly low cost. Different media initially prepared with different combination and concentration of gelling agents to know the best gelling agent among the four used in the study (i.e. rice starch, cassava, sago and pectin). Both solid and liquid media were formulated. All the media formulated produced good growth of microbes similar to the conventional media, but microbial growth was best in both solid and liquid culture media when provided with peptone and beef extract at $1/4^{th}$ concentration compared to conventional synthetic media. By the addition of only $1/4^{th}$ concentration of commercially available nutrient sources, the cost of the routinely used media can be reduced to a remarkable extent. The study yields good clue for exploring important bio-products for developing new essential growth media which will be cheap and easily available.

Keywords: Local food substrate, conventional media, gelling agent, bio-product.

Introduction: Much of the study of microbiology depends on the ability to grow and maintain microorganisms in the laboratory, and this is possible only if suitable culture media are available. 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 thus while culturing bacteria, it is very important to provide similar environmental and nutritional conditions to organisms [1].

Most often, a culture medium contains water, a source of carbon & energy, source of nitrogen, trace elements and some growth factors [2]. The cost of dehydrated media is very high and due to increasing cost of agar, it has become a bane to microbiologists, particularly in developing countries where culture media are imported and budgets are unavoidably low. Therefore it is very much essential to look out for alternate efficient but cheaper media.

Agricultural wastes are useful substrates for production of microbial protein and plant materials have been used to recover both fungi and bacteria using Groundnut, Sorghum extracts, local food stuff waste, cassava whey, three – leaf yam, African oil bean, maize, beans and pigeon pea [3].Such plant material can be used as source of nutrients like source of carbon and energy or/and nitrogen or/and growth factors. They must be non toxic, abundant, totally regenerable, non-exotic, cheap and able to support rapid growth and multiplication of the organisms resulting in high quality biomass

[4]. Similarly, agar is the most widely used gelling agent for culture media because of its stability, high clarity, nontoxic nature, and resistance to metabolism. In the recent past, due to exorbitant price of bacteriological grade agar, doubts about its inertness and nontoxic nature and fear of overexploitation of its sources potential of various gelling agents have been evaluated. For e.g. agarose, xanthan gum, gellan gum, guar gum, GELRITE, carrageenan, starch and gelatin etc [5]. Based on the market value and the scarcity of culture media, screening of alternate media is found to be an important task.

Materials and Methods:

Collection of culture isolates:

Standard bacterial cultures namely *E. coli, S. aureus, P. aeruginosa, Kl. pneumonia, P. vulgaris, S. typhi* and yeast *C. albicans* were used.

Collection of samples:

Agricultural yields like Tomato, Carrot, and Spinach, and soya bean were purchased from local market. The collected samples were transported to the laboratory and processed immediately. The gelling agents like Tapioca (cassava) starch, rice flour, Sago flour, and pectin procured from local market were used. The starch was extracted by steeping grated tapioca in sterile distilled water for 4-5 days, supernatant decanted to obtain clear starch paste, which was oven dried at 120˚C for 45 mins and crushed into fine powder. Gelling agents at various concentrations were added to the basal nutrient medium and heated for 15 to 20 min to get good solution (Table 1). Plates containing agar as the only gelling agent were used as control. Gelling ability was assessed only by rotating the Petri dish containing the medium at different time intervals. Gelling agents yielding promising results were then used in preparation of solid test media for microbial growth studies.

Formulation of media:

The fresh raw vegetables (Carrot-500g, Tomato-500g, Spinach-250g) were manually cut and crushed using electronic blender to extract the fluid. The extract was then filtered using suitable filters. Approximately 300ml, 400ml, 250ml extract was obtained respectively, which were then transferred to sterile 500ml flask. These extracts were used at concentration of 20% (v/v) in various media formulations. Soya bean (250 g) was grounded to obtain soya bean flour. The flour was stored in clean bottle till use.

Four different media formulations (100 ml) were prepared using distilled water as diluent. 1) by mixing only the extracts (20% v/v) of fresh raw vegetables and soya bean flour (20 gm %). 2) extracts (20% v/v) were mixed with beef extract (0.07gm%) 3) extracts (20% v/v) were mixed with peptone (0.2gm%) 4) extracts (20% v/v) with both beef extract (0.07gm%) and peptone (0.2gm%). (Table 1). (The concentration of beef extract and peptone selected was nearly 1/4th of that present in commercial dehydrated media which was used as control media). The pH was maintained at 7 ± 0.5 for bacteria and 5 ± 0.5 for fungi. The media was sterilized at 121 ºC for 15 minutes.

Solid media formulation:

An appropriate amount of alternate agar derivative was added in the formulated media (Table 1) and 15ml of the sterilized medium was distributed into each of the sterile petri dishes. Plates were prepared with different concentrations of various gelling agents like rice flour, cassava starch, sago flour and pectin as the only solidifying agent (ie 4, 8, 12, 16 and 20gm% of gelling agent). Also plates were prepared using 1gm% agar in combination with various gelling agents at concentrations of 2.5, 3.5, 4.5, 5.5 and 6.5gm%.

A loop ful of each test culture was streaked on each test media and incubated at 37˚C/ 24-48hrs for bacterial cultures and at R.T/ 48hrs for fungal culture. After the incubation period,

plates were observed and the colony sizes were recorded. The degree of growth was then compared with the control media (Nutrient agar- bacterial culture, Sabouraud's agar- fungal culture).

Liquid media formulation:

The liquid media was prepared according to Table 1. Optical Density of 24h old cell suspension cultures was adjusted to 0.1 and then inoculated in all the formulated broths. Absorbance was measured at 540nm at the end of incubation period ie 37˚C /24 hrs for bacteria and at R.T/48hrs for yeast. The bacterial control was maintained in Nutrient broth and the fungal control in Sabouraud's broth for comparison. Since the soybean broth was turbid, viable count was performed instead of O. D.

Table 1: Different solid and liquid media formulations

Media	**Ingradients (for 100ml of media)**	**Different combinations of media**			
		1	**2**	**3**	**4**
Tomato	Beef extract (gm)	-	0.07	-	0.07
	Peptone (gm)	-	-	0.2	0.2
	Tomato Extract (ml)	20	20	20	20
Carrot	Beef extract (gm)	-	0.07	-	0.07
	Peptone (gm)	-	-	0.2	0.2
	Carrot Extract (ml)	20	20	20	20
Spinach	Beef extract (gm)	-	0.07	-	0.07
	Peptone (gm)	-	-	0.2	0.2
	Spinach Extract (ml)	20	20	20	20
Soyabean	Beef extract (gm)	-	0.07	-	0.07
	Peptone (gm)	-	-	0.2	0.2
	Soyabean flour (gm)	20	20	20	20

1= media with only tomato /carrot /spinach /soyabean flour

2= media with extract + beef extract

3= media with extract + peptone

4= media with beef extract + peptone

The results were noted by carrying out the experiment in duplicates.

Results 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. With increasing market value of readymade, dehydrated media, screening of alternate media is found to be an important task [6].

Hence an alternative media using fresh raw vegetables (Carrot, Tomato and Spinach) and soya bean, was designed. The ability of different formulations of both solid and liquid media (Table-1) to support the growth of the test organisms in comparison to the growth on conventional media was investigated and similarly the gelling performance of various potential gelling agents was also investigated.

Different media prepared with different combination and concentration of gelling agents to know the best gelling agent among four natural gelling agents used in study (i.e rice starch, cassava, sago and pectin) on the basis of binding and gelling ability of these natural products which was assessed only by rotating the Petri dish containing the medium after 24 h.

Among agar substitutes tested, tapioca starch at minimum 5.5gm% and sago flour at 4.5gm% with the addition of 1gm% agar were the gelling agents that gave promising results within 10min. Other gelling agents like rice and pectin were either runny or very sticky or so thick that they formed lumps soon after autoclaving and well before pouring the media into Petri plates. The plates fortified with tapioca and sago, on the other hand, were rock solid with clear consistency but none of these gelling agents alone even at higher concentration (20gm%) could gel. Agar was added only to form a stable surface to streak on because even though the gelling agents solidified they were sticky to streak upon. Similar results were obtained when cassava starch was tested for its gelling ability by C.K. Kwoseh, M. Asomani-Darko and K. Adubofour [7] and Mbanaso *et al.* (2001) [8]. This is probably due to drop in pH that reduces gel stability which often occurs after autoclaving the media (Lupano and Gonzalez, 1999) [9] . Dabai *et al* in 2005 studied use of Cassava starch powder as a solidifying agent in microbiological nutrient media and reported it as a potential solidifying agent in microbiological nutrient media as an alternative to agar-agar[10].

Therefore none of the agents can completely substitute agar but they can be used in combination (5.5gm% tapioca starch & 1gm% agar or 4.5gm% sago flour & 1gm% agar) to reduce the cost of media. Both tapioca and sago are widely available and are cheap. 345.96gms of tapioca starch was extracted from 1kg of tapioca tuber (49 Rs) and sago costs rupees 64/kg whereas 500gm of agar can cost nearly about Rs 3800/-. Besides having a cost advantage, they pose no problem in adjustment of pH and dispensing.

Considering cost effectiveness and gelling property, further study using solid media was done with Cassava starch as solidifying agent at optimum concentration (5.5gm%) with 1% agar in various media. When different organisms were streaked on various plates with extracts of Tomato, Spinach, Carrot and Soya bean as nutrients (as given in table 1), average colony size (in mm) of different organisms was as given in Table 2.

All the solid media formulations, containing only nutrient substitutes i.e. carrot, tomato, spinach extracts and soybean flour as the sole source of nutrient were able to support the growth of all the test organisms suggesting that all the nutrient substitutes used contains enough nutrients (Table 2) to allow the growth of microorganisms. The growth of test organism was either same or less in terms of colony size (mm) on the test media with only extract as compared to the control media. It may be due to unavailability of simple, easily metabolizable source of protein. Therefore, the formulated media was supplemented with beef extract

and/or peptone at 1/4th of its concentration as compared to that in control media. After the addition of beef extract and peptone, all the test organisms showed enhanced growth. The growth response was nearly double for *E. coli, K. pneumoniae* and *S. typhi* on addition of tomato extract along with beef extract and peptone at low concetration ie at 1/4th concentrations as compared to control media. Similar response was observed for *E. coli, S. aureus, K. pneumoniae* and *S. typhi* on media with carrot extract, spinach and soyabean extract. *C albicans* showed remarkably enhanced growth in presence of spinach extract.

For the investigation of liquid media formulations, only 3 cultures namely *E. coli, S. aureus* and *C. albicans* were used. Results were noted in terms of O. D at 540 nm and were as given in table 3. Broth prepared using soybean flour was dense and unclear, therefore viable count was performed.

Broth with tomato extract and carrot as the only nutrient supplement was not good for growth of *E. coli* and *S. aureus* test organisms. Substantial increase in the growth was observed for *S. aureus* and *E. coli* on addition of beef extract and peptone at concentration 1/4th to that present in control broth media.

Addition of spinach extract alone was found to be the best growth enhancer for all three test cultures *E. coli, S. aureus* and *C. albicans* with O.D. 1.57, 1.17 and 1.40 respectively.

All three extracts (tomato, carrot and spinach) were excellent growth enhancer for *C. albicans* even when used alone as source of nutrients. Also further marked increase was observed with addition of beef extract and peptone even at low concentrations. Spinach extract was found to be the best for growth of bacteria as well as yeast.

Soya bean extract was good only for the growth of bacteria when used alone as the source of nutrients as well as when used in combination with beef extract and peptone. But yeast showed reduced growth in presence of soybean extract.

Deivanayaki M and A Iruthayaraj in 2012 also reported good growth of bacteria on media formulation using varying conc. of cabbage, tomato, carrot and pumpkin. They reported growth as *Staphylococcus sp*., 230 CFU / ml in Formulation E (0.3g carrot+0.5g cabbage+0.001gNaCl), 150 CFU / ml of *Escherichia coli* in Formulation A (0.3g carrot+0.25g tomato+0.25 cabbage+0.001gNaCl), 170 CFU / ml of *Salmonella sp.,* in Formulation B (0.15 carrot+0.25g tomato+0.25g cabbage+0.001gNaCl) , 150 CFU / ml of *Klebsiella sp.*, in Formulation B (0.15 carrot + 0.25g tomato + 0.25g cabbage +0.001gNaCl) [11].

Ravathie Arulanantham *et al,* in2012 formulated media using edible leguminous seeds such as green gram, black gram; soya meat and cowpea to find the feasibility of using legume seeds as an alternative nutrient source to grow bacteria. It was reported that the different protein sources with varying proportions of agar have different solidification times. The formulated media consisting of protein source and agar supported the growth of all test organisms *Klebsiella, Bacillus* and *Staphylococcus*[12].

Similarly G.A. Płaza *et al.*, (2011) reported that the three *Bacillus* strains [Bacillus subtilis (I′-1a), Bacillus sp. (T1), Bacillus sp. (T′-1)] can grow on various waste products including vegetable decoction as organic media [13] . **Abalaka, M.E** *et al* in 2013 prepared nutrient agar using guinea corn and maize and compared with commercially prepared nutrient agar. They suggested that, locally prepared media from local substrate such as guinea corn, maize and potato could be used in the absence of commercial prepared nutrient media and potato dextrose agar[14].

Table 2: Colony sizes of well-isolated colony of test cultures on various agar media.

Culture	Average diameter of colony (mm) on various media with								
	Control	Tomato		Carrot		Spinach		Soyabean	
		1	2	1	2	1	2	1	2
E. coli	< 1.0	<1.0	2.0	<1.0	2.0	1.0	2.0	1.0	>1.0
S. aureus	1.0	<1.0	>1.0	1.0	2.0	1.0	2.0	1.0	2.0
P. aerugenosa	< 1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
K. pneumoniae	< 1.0	<1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0
P. vulgaris	< 1.0	<1.0	1.0	<1.0	1.0	<1.0	1.0	<1.0	1.0
S. typhi	< 1.0	<1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0
C. albicans	< 1.0	<1.0	1.0	<1.0	1.0	1.0	2.0	1.0	1.0

1= media with only extract, 2= media with extract+ beef extract+ peptone

Control media= Nutrient agar and Sabouraud's agar

Table 3: Growth (in terms of OD or cfu/ml) of test cultures in various broth media.

Culture	Average optical density at 540 nm							Average cfu/ml		
	Control	Tomato		Carrot		Spinach		Control	Soyabean	
		1	2	1	2	1	2		1	2
E. coli	1.07	0.80	1.39	0.75	1.02	1.57	2.03	$8.3x\ 10^9$	$1.3x10^{10}$	$3.1x10^{10}$
S. aureus	0.70	0.59	1.23	0.53	1.15	1.17	1.90	$1.0x10^{10}$	$2.45x10^{10}$	$4.3x10^{10}$
C. albicans	0.35	1.03	1.47	0.96	1.28	1.40	2.65	$7.4x10^9$	$2x10^9$	$3x10^9$

1= media with only extract, 2= media with extract+ beef extract+ peptone

Control media= Nutrient agar and Sabouraud's agar

Conclusion: From the results it can be concluded that nutrients obtained by plants, vegetables, cereals, legumes etc can be a good substitute for microbial culture media. For better results, such media may be supplemented with commercially available nutrient sources at low concentrations. Just by the addition of only $1/4^{th}$ concentration of other commercially available nutrient sources, the cost of the routinely used media in laboratories and industries can be reduced to a remarkable extent. The use of such rich, inexpensive, potential source of raw material not only boosts the growth response of microorganisms but will also make the disposal of these materials easy, beneficent to environment.

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Immanuel Rebecca /Department of Microbiology/ R K Talreja College/Ulhasnagar/ Dist Thane-421203, India, Post grad student/University of Mumbai/ rebeimmanuel@gmail.com

Patil Rohini Pradeep /Department of Microbiology/ R K Talreja College/Ulhasnagar/ Dist Thane-421203, India/Associate professor/ Affiliated to university of Mumbai/ rohini_106@yahoo.com.