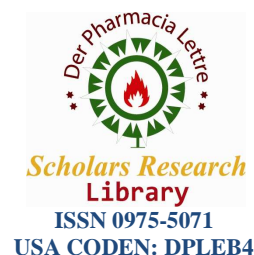




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## Formulation of alternative culture media for bacterial and fungal growth

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### ABSTRACT

*The microorganisms are grown for many purposes. Culture media used in the laboratory for the cultivation of microorganism supply the nutrients required for the growth and maintenance. Nutrient agar (NA) is universally used as a general purpose medium for the cultivation of broad range of bacteria. Potato Dextrose Agar (PDA) is commonly used for the isolation and growth of wide range of Fungi. The feasibility of developing alternative media to different culture media namely PDA and NA were assessed using locally available cheap materials because the use of readymade culture media in schools and laboratories has financial limitations. Generally the cheap locally available materials such as cereals and legumes may serve as alternative nutrient media to grow bacteria and fungi. The materials used in this study were rice, chickpea, corn, dhal, thinai, natural soy flour and processed soy flour (TVP). For bacteria the test organisms used were E.coli, Pseudomonas sp., Bacillus sp., Staphylococcus sp. and Klebsiella sp. The Klebsiella sp. grows well in all alternative nutrient sources and generally Bacillus sp., grows less in all alternative culture media. The colony morphological characters of bacteria such as shape, margin, elevation and colour were generally similar compared with NA. For fungi the test organisms used were Trichoderma sp., Aspergillus sp., Penicillium sp., Sclerotium sp. and Fusarium sp. On average Sclerotium sp. showed significantly ( $p < 0.05$ ) higher growth in rice. Penicillium sp. showed significantly ( $p < 0.05$ ) low growth in rice and corn. The present study clearly showed in comparison with PDA and NA the possibility of using cheap locally available materials such as rice, chickpea, corn, dhal, thinai, natural soy flour and TVP as alternative nutrient media for bacteriological and mycological studies.*

**Key words;** culture media, alternative nutrient sources, NA, PDA, bacteria , fungi

### INTRODUCTION

Microbiological studies depend on the ability to cultivate and maintain microorganisms under laboratory conditions by providing suitable culture media that offer favorable environmental conditions. A nutrient material prepared for the growth of microorganisms in a laboratory is called culture media. 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 and water.

NA medium is commonly used as general purpose medium for the cultivation of broad range of bacteria. It is a basic medium composed of peptic digest of animal tissue, beef extract, yeast extract, sodium chloride and agar (HEIMEDIA).

Fungi are a group of eukaryotic spore bearing microorganisms. They generally reproduce asexually and sexually. Some are agents of diseases in plant and animals (parasitic) while some are saprophytic and play a major role in nutrient recycling. PDA is commonly used for the growth of fungi in laboratories and composition is well defined. PDA contains potato infusion, dextrose and agar (HIMEDIA)

Those readymade media PDA and NA are used for the growth of microorganisms but these are expensive to be used in schools. Microbiological researches are carried out at high cost and scarcity of culture media (Adesemoye and Adedire, 2005). It is one of the serious problems for a developing country. Microbial researches are hindered by high cost of culture media. Therefore we have to try to use various alternative media and agar to reduce the cost involved. Agar is a solidifying agent and very few studies have concentrated on replacing agar for solidification. In line with the other studies carried out in this area by a number of authors, this group (Ravathie *et al.*, 2012; Nirmala *et al.*, 2014) has worked on the possibility of using a number of sources as alternative culture media. Even then, there is a necessity to formulate new media with easily available low cost material as substitutes for NA and PDA.

The present study is aimed at replacing the nutrient source by various locally available cheap materials such as cereals and protein sources that contain considerable amount of protein and starch. The nutrient materials used commonly constitutes Sri Lankan food. These good nutrient source media are cheap and easily available in local shops and supermarkets. They were selected as a natural nutrient source to prepare the alternative culture media.

## MATERIALS AND METHODS

### COLLECTION OF SAMPLES

The tested alternative nutrient samples such as Rice (*Oryza sativa*), Chickpea (*Cicer arietinum*), Corn (*Zea mays*), Dhal (*Lens culinaris*), Thinai (*Setaria italica*), Natural soy flour and Processed Soy flour (TVP) (*Glycine max*) were purchased from local shops in Jaffna. They were identified and confirmed by a taxonomist in the Department of Botany, University of Jaffna.

### SOLID MEDIA FORMULATION

The required sample was completely ground into fine powder by electric blender (National) with the use of sieve. Then the powdered samples were kept in air tight containers until its use. Seven different solid media were prepared as follows. Specific amount of sample from each was taken and mixed with (0.5 - 4 g) of agar (HIMEDIA). The solidification times of the alternative nutrient samples were found out. According to the results finally it was decided to use 1 g of agar dissolved in 100 ml of distilled water with 3 g of each sample powder. PDA and NA plates served as control. In all experiments the pH of the media was measured and adjusted to  $7 \pm 0.2$ . The dissolved media were sterilized by an autoclave (Dixons, ST19T) at  $121^{\circ}\text{C}$  for 20 minutes under the pressure of 15 lbs/inch<sup>2</sup> and were poured into sterile petridishes separately.

### PREPARATION OF FRESH CULTURE BACTERIA

The tested bacterial samples such as *E.coli*, *Pseudomonas* sp., *Bacillus* sp., *Staphylococcus* sp. and *Klebsiella* sp. were collected from the bacterial culture collection of the Department of Botany, University of Jaffna. Then these bacteria were streaked on the freshly prepared NA medium from the original stock culture. The cultures were allowed to incubate at  $37^{\circ}\text{C}$  for 24 hours.

### FUNGI

In this study five different fungi namely *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp., *Sclerotium* sp. and *Fusarium* sp. were obtained from the fungal culture collection of the Department of Botany University of Jaffna. Prior to the study above fungal disks were put into fresh PDA medium. The cultures were incubated at room temperature for 2-3 days.

### MICROBIAL INOCULATION INTO ALTERNATIVE MEDIA SOURCES. BACTERIA

The young cultures of tested bacteria such as *E.coli*, *Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp., and *Klebsiella* sp. were taken. Serial dilution was done according to the standard method to get a final bacterial inoculum concentration of  $1.0 \times 10^8$  cell/ml. 0.1 ml bacterial suspension was taken using a sterile pipette. It was inoculated on to the center of solid media under sterile conditions and was spreaded uniformly by a sterile glass

spreader. The same bacteria were inoculated in triplicates in each alternative culture medium. Then all the plates were incubated at 37°C for 48 hours. After the incubation all the plates were observed for bacterial growth and the number of colonies was counted in the triplicate plates.

## FUNGI

Actively growing pure culture of test fungi such as *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp., *Sclerotium* sp. and *Fusarium* sp. were taken. Then a fungal disc was cut by using 8 mm sterile cork borer and placed on the surface of each alternative nutrient culture media in replicates. The tested fungi were also introduced on PDA media which served as control. Then all the plates were incubated at room temperature for 2-3 days. After incubation diameter of fungal mycelia was measured by using a ruler in 3 directions and then average diameter was calculated for each fungus.

## STATISTICAL DATA ANALYSIS

All data were statistically analyzed by STATISTICA (p<0.05) software (version 6;statsoft Inc.,Tulsa,USA).

## RESULTS AND DISCUSSION

### SOLIDIFICATION TIME

The results showed various solidification times (Table: I) when different alternative nutrient sources were mixed with varying proportions of agar ranging from 0.5 g - 4 g. However similar solidification time for nutrient broth + agar was obtained when 1.5 g - 2 g of agar was added to different protein sources, which is consistent with the results obtained by Deivanayaki and Iruthayaraj (2012) for vegetable source and Annan-Prah *et al.* (2010) for Cowpea. 3 g of sample was taken in the experiment which was carried out by Revathi *et al.* (2012). In our experiment 1 g of agar was used with 3 g of sample, because in our experiment the purpose is to reduce the amount of agar, as the price of agar is high so in this case where the reasonable working time changes i.e. increase or decrease.

Even though the pH values were kept constant at 7 throughout the experiment it was our view to check whether these alternative nutrient samples can be used without adjusting the pH as many of the schools in developing countries lack pH meter. The pH values of different formulations ranged from 6.12 - 6.89. It was interesting to note that there was no significant change in the diameter of growth whether the pH was adjusted to 7.0 or when the experiment was carried out as such.

**Table I: Solidification time of various nutrient sources**

Weight of sample(g)	Weight of agar (g)	Rice (minutes)	Chickpea (minutes)	Corn (minutes)	Dhal (minutes)	Thinai (minutes)	Natural Soy Flour (minutes)	TVP (minutes)
3.0	0.5	57	55.0	56	65	68	62	68
3.0	1.0	38	35.0	34	36	37	30	41
3.0	1.5	35	20.0	26	24	28	26	37
3.0	2.0	27	20.0	23	21	26	25	31
3.0	2.5	24	18.5	21	20	25	20	27
3.0	3.0	23	14.0	20	18	24	19	24
3.0	3.5	19	15.0	17	15	23	18	23
3.0	4.0	17	9.0	16	14	20	13	18
NA	3.0	18	19.0	19	17	19	20	20

The alternative culture media such as rice, chickpea, corn, dhal, thinai, natural soy flour and TVP contain different nutrient sources. Agar is a solidifying agent. It is used to solidify the alternative culture medium. After the solidification of medium, it is easy to spread the inoculum and we can easily calculate the number of colonies in solid media.

### BACTERIA

Among the five bacterial inocula, *Klebsiella* sp., showed significantly (p>0.05) high growth rate in chickpea, while less growth rate in rice (Figure 1). In most of the alternative nutrient sources *Klebsiellas* sp., showed significantly (p>0.05) high growth rate than other tested inocula except in soy flour. *Bacillus* sp., showed significantly (p>0.05) high growth in NA next to chickpea and showed less growth rate in thinai. *Pseudomonas* sp., showed significantly (p>0.05) high growth rate in natural soy flour and showed less growth rate in rice.

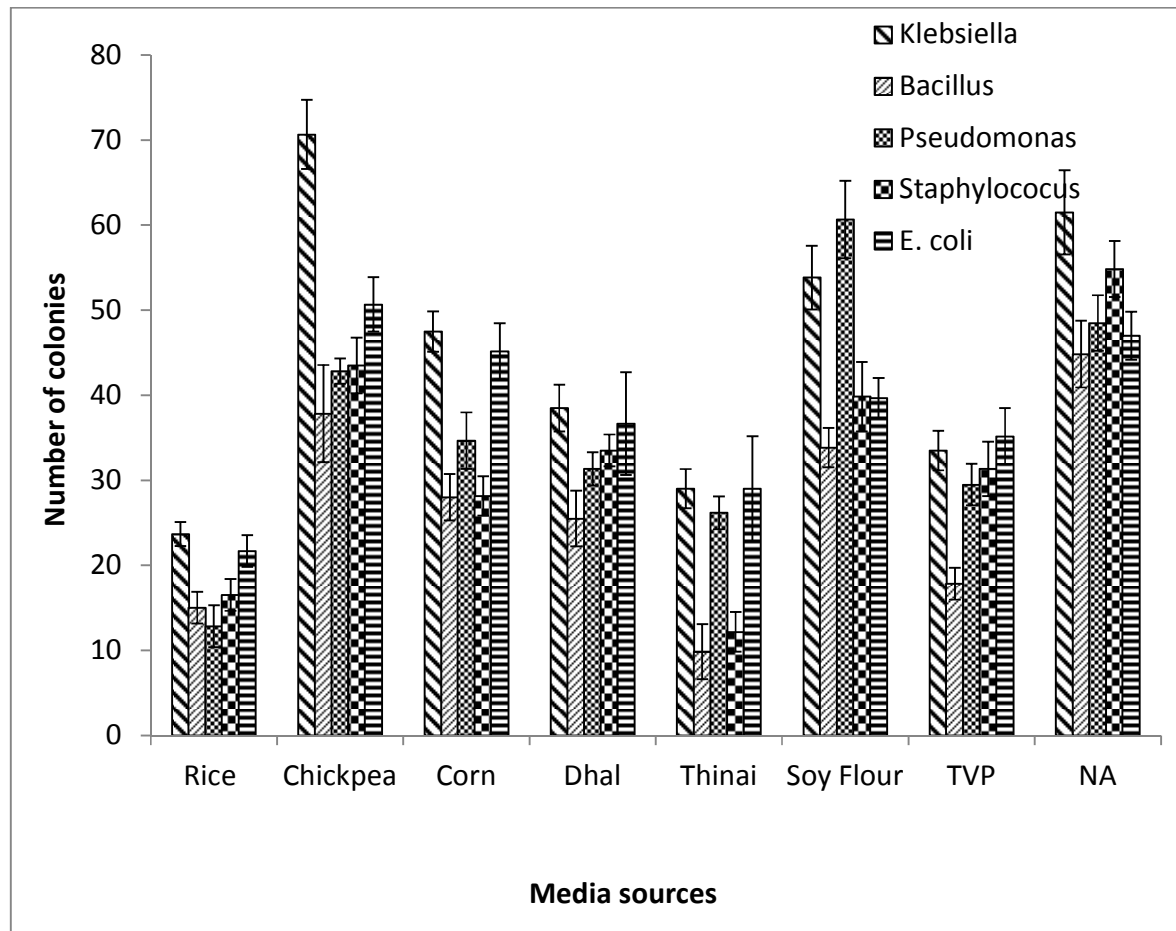


Figure 1 bacterial growth in different alternative formulations

Other test organism *Staphylococcus* sp., showed significantly ( $p>0.05$ ) high growth rate in NA next to chickpea and showed less growth rate in thinai. *E.coli* showed significantly ( $p>0.05$ ) high growth rate in chickpea and showed less growth rate in rice.

It is interesting to note that *Klebsilla* sp. showed significantly ( $p>0.05$ ) high growth rate in chickpea than NA. *Pseudomonas* sp. showed comparatively high growth rate in natural soy flour than NA.

When each of the test organism was grown in various alternative nutrient sources various results were obtained. All the test organisms showed significant growth in all protein sources but comparatively less growth in rice.

There are a number of studies concentrated to screen alternative source of culture media. To replace NA plant materials have been used for the growth of bacteria from different sources. Another study on growth and cultural characteristics of selective bacteria on cowpea agar was done by Annan Prah *et al.* (2010). In a study sago was used as alternative culture media (Kapilan and Thavaranjit, 2008). The legume seeds were also used as alternative culture media (Ravathie *et al.*, 2012).

NA is used as a common culture medium to grow various bacteria. This consists of nutrient broth and agar. The cost of 1kg of nutrient broth (BIOCHMIKA) is approximately € 93 (15 750 LKR). It costs around 95 LKR to prepare 1 liter of NA medium whereas it costs less than 1 LKR to prepare different alternative formulations. Thus the use of different alternative formulations as culture media in laboratories with basic facilities is very much feasible and cheaper when compared to commercially prepared NA. Although these alternative formulations can be prepared instantly, they can even be stored for more than a month at room temperature in tropical climate.

## FUNGI

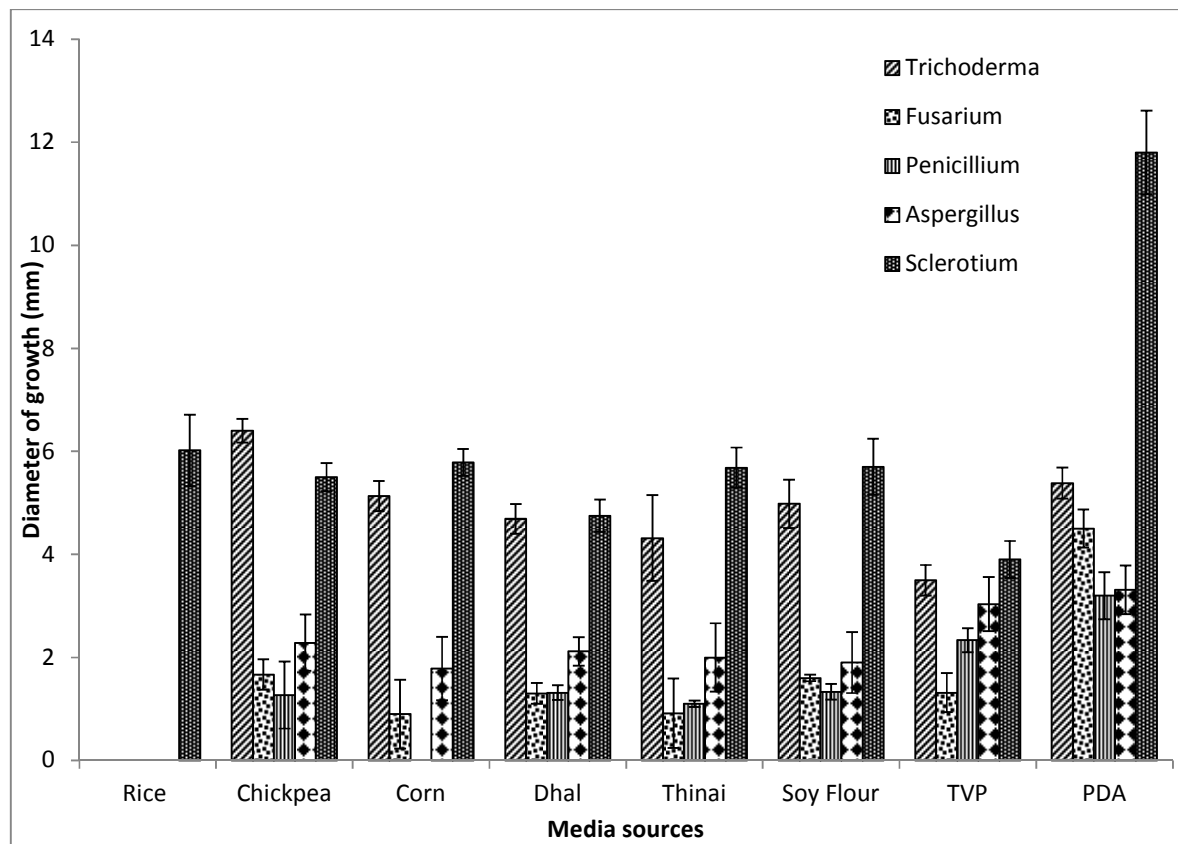


Figure 2-fungal growth in different protein formulation

In this experiment the test organisms such as *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp., *Sclerotium* sp. and *Fusarium* sp. showed significantly higher growth in all nutrient formulation medium but comparatively less growth was observed in rice (Figure 2). *Sclerotium* sp., *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. showed significantly ( $p > 0.05$ ) high growth rate in PDA than other alternative culture media. But *Trichoderma* sp., showed high growth rate in chickpea than PDA. It is interesting to note that only *Sclerotium* sp. grows in rice.

Among the five tested organisms *Fusarium* sp., *Penicillium* sp., *Sclerotium* sp., and *Aspergillus* sp., showed significantly ( $p > 0.05$ ) high growth rate in PDA, and less growth rate in rice, except *Sclerotium* sp. which showed less growth rate in TVP.

On the whole, *Sclerotium* sp. showed high growth rate in all the alternative culture media except in chickpea as in the case with PDA. *Penicillium* sp. showed comparatively less growth rate in all alternative culture media.

There are a number of studies concentrated to screen alternative culture media to replace PDA. One study was aimed to determine the optimum extraction process for hom mali brown rice flour (HMBRF) and to investigate the growth of *Aspergillus niger* using the extract as culture media (Porndarun *et al.*, 2010). Another study was done on preliminary screening of alternative culture media for the growth of some selective fungi (Tharmila *et al.*, 2011). In this study an attempt was made to test the possibility of using some cheaply available materials (sago, raw dried Palmyrah tuber flour) as alternative media for fungal growth.

PDA is used as a common culture medium to grow various fungi. The cost of 1 kg of PDA (biochmika) is approximately \$ 100 (12 750 LKR). It costs around 400 LKR to prepare 1 liter of PDA medium whereas it costs approximately 300 LKR to prepare same volume of different alternative formulations. Thus the use of different

alternative formulations as culture media in laboratories with basic facilities is very much feasible and cheaper when compared to commercially prepared PDA. Although these alternative formulations can be prepared instantly, they can even be stored for more than a month at room temperature in tropical climate.

### CONCLUSION

Based on this study it is concluded that *Klebsiella* sp. grows well in most of the nutrient/media sources including NA with maximum growth in chickpea. *Bacillus* sp. generally grows least in all the nutrient sources except in rice. Among the different tested sources *Klebsiella* sp. and *Pseudomonas* sp. showed significantly ( $p < 0.05$ ) higher growth rate in Natural soy flour than NA. All the test organisms except *Pseudomonas* showed significantly ( $p < 0.05$ ) high growth rate in chickpea.

It should be noted that this study has used a common bacterium *E. coli* along with the other bacteria. It is important to note that *E. coli* is generally used in microbiological studies in almost all labs. Studies should be carried out to find the chemical compositions of alternative nutrient sources and biochemical characteristics of the bacteria in different alternative nutrient sources and more test organisms (bacteria) should be used to identify the suitability of using these alternative nutrient sources as alternative general purpose medium. It is recommended that further research be conducted with other possible alternative nutrient sources for the production of bacterial culture medium.

Based on the findings of the study it is concluded that *Sclerotium* sp. showed significantly ( $p < 0.05$ ) high growth rate in all alternative culture media which is consistent with the growth in PDA. *Trichoderma* sp., showed significantly ( $p < 0.05$ ) highest growth rate in chick pea among the nutrient sources used in this study. In all tested alternative sources *Penicillium* sp. exhibited significantly ( $p < 0.05$ ) less growth rate in chickpea and soy flour and virtually no growth in rice and corn. *Aspergillus* sp., showed significantly ( $p < 0.05$ ) high growth rate in TVP among the alternative nutrient sources which compares very well with PDA.

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