Available online at www.scholarsresearchlibrary.com

Scholars Research Library

***J. Nat. Prod. Plant Resour.*, 2012, 2 (6):697-700**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Alternative culture media for bacterial growth using different formulation of protein sources

Ravathie Arulanantham, Sevvel Pathmanathan, Nirmala Ravimannan and Kularajany Niranjan

Department of Botany, Faculty of Science, University of Jaffna. SriLanka.

ABSTRACT

The exorbitant costs of culture media have deprived the use of readymade culture media such as nutrient agar in schools and laboratories with less facility. Generally legume seeds are found to be a good protein source for nutritional purposes. This study was carried out to find the feasibility of using legume seeds as an alternative nutrient source to grow bacteria. Cowpea, green gram, black gram and soya meat (processed soya bean) were used in this study. The test organisms used were E. coli, Bacillus sp., Klebsiella sp., Staphyllococcus sp. and Pseudomonas sp. Staphyllococcus sp. grows well but slowly (332 – 356 CFU/0.1ml) and generally Klebsiella sp. (196 - 232 CFU/0.1 ml) grows least in all the protein formulations tested. All the tested bacteria grow least in green gram. In comparison with the performance on conventional nutrient agar media, the prepared protein formulations were found to be cheap and good alternative culture media for bacteriological studies.

Keywords: Culture media, bacteria, protein formulation, nutrient agar

---

INTRODUCTION

Nutrient Agar is a culture medium recommended for the cultivation of non-fastidious microorganisms. Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. Culture media used in the laboratory for the cultivation of microorganisms supply the nutrients required for growth and maintenance. Nutrient agar is a common medium used to grow bacteria in laboratories. This is a basic medium composed of a simple peptone and a beef extract. As the readily available culture media are expensive, there is a need to find alternative media or reduce the amount of agar added during the preparation of culture media in laboratories with less facility. Arnan-Parh et.al has worked on cowpea as a cost effective alternative culture media [1]. Though the authors have used cooked (boiled) cowpea to increase the shelf life up to three months in their study, we presume that it is not essential to boil as it is readily available. There are reports that used starch as less expensive growth media for bacteria and fungi [1, 2]. Further there are also reports using vegetables as an alternative source for preparing culture media [4].The present study is aimed at replacing the nutrient source by a protein formulation. Legume seeds serve as a good protein source and they are locally available cheap materials. Green gram, black gram, cowpea and soya meat were selected as a natural protein source to formulate the media.

MATERIALS AND METHODS

Collection of samples

Edible leguminous seeds such as green gram, black gram, soya meat and cowpea were purchased from shops in Jaffna and their identity was confirmed using a taxonomist in the University.

Solid media formulation

The samples were finely powdered separately using electric blender and sieved. The powder was stored separately in sterile containers until its use. Four different solid media were prepared as follows. 3g from each protein source was taken and mixed with 0.5-3.0 g of agar (HIMEDIA). The solidification times of each media preparation with 0.5-3.0 g of agar were recorded.

Finally 3g of Agar (because this is the amount used in HIMEDIA Nutrient agar and seems to have a reasonable working time) was added and dissolved in 100ml distilled water with different protein sources in all the experiments. The pH of the media was measured and adjusted to 7 ± 0.2.

Serial dilution

The bacteria (*E.coli, Bacillus* sp., *Klebsiella* sp., *Staphyllococcus* sp. and *Pseudomonas* sp.) were collected from the microbial culture collection in the Department of Botany, University of Jaffna. Then these bacteria were streaked onto nutrient agar medium. The cultures were allowed to incubate at 37 ºC for 24 hrs. Serial dilutions of the bacteria were prepared using the standard procedure using saline water (0.85 %).

Microbial Inoculation

The standardized culture (0.1ml of overnight culture) of each test bacteria namely *E.coli, Bacillus* sp., *Klebsiella* sp., *Staphyllococcus* sp. and *Pseudomonas* sp. were inoculated onto the solid media in triplicates by spreading. The initial inocula used were approximately 2X107cell/ml. The organisms introduced on nutrient agar media served as control. The inoculated plates were incubated at 37 ^{0}C for 24-48 hours. After incubation the plates and respective controls were observed for the degree of growth in terms of number of colonies.

Statistical data analysis

The number of colonies obtained for each protein formulation as well as the specific bacterium growing on different protein formulations were analyzed by analysis of variance (ANOVA) ($P<0.05$) followed by LSD by using software MINITAB.

RESULTS AND DISCUSSION

The results showed that when the different protein sources with varying proportions of agar have different solidification times as expected. However similar solidification time for nutrient broth+agar was obtained when 1.5-2.0 g of agar was added to different protein sources which is consistent with the results obtained by Deivanayaki and Iruthayaraj for vegetable sources and Annan Prah et al. for cowpea alone [4, 1]. This means that less amount of agar can be used with the alternative protein sources reducing the amount of agar used even though in this study 3g agar is used. However if the amount of agar used is increased by one fold, approximately there is a tenfold reduction of time duration taken for solidification (Table 1.1). So in those cases, where the reasonable working time changes i.e. increase or decrease, the amount of agar added can be changed accordingly.

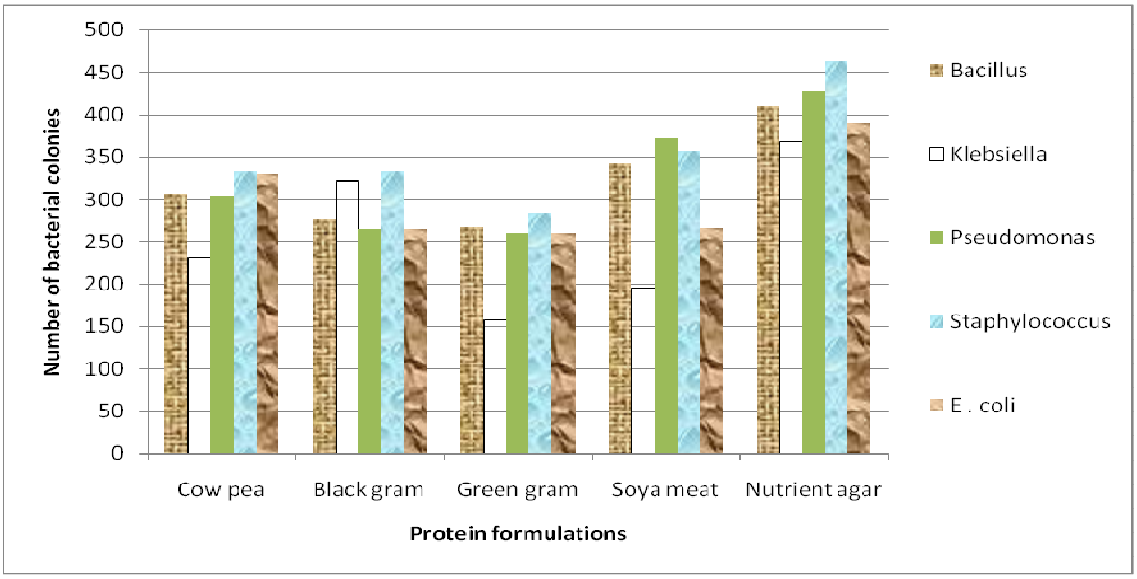
Table 1.1: Solidification times of various protein sources with different proportions of agar

Weight of protein sources(x)g	Weight of agar(Y)g	Soya meat (mean setting time)(min)	Black gram (mean setting time)(min)	Green gram (mean setting time)(min)	Cowpea (mean setting time)(min)
3	1	>43	42	43	42
3	1.5	37	41	33	30
3	2	33	34	30	24
3	2.5	31	33	28	22
3	3	22	18	22	20
3	3.5	16	14	18	17
3	4	12	12	13	12
3 (Nutrient broth)	3	36	38	31	30

In each case, the mean value was obtained from the experiment carried out in triplicates
X –weight of cowpea, soya meat, black gram and green gram.
Y -Weight of agar powder.

Even though the pH values were kept constant at 7 throughout the experiment, it was our view to check whether these protein formulations can be used without adjusting the pH as many of the schools in the developing country may not have a 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 number of colonies whether we adjusted the pH to 7.0 or went on with the experiments as such.

Fig.1: Bacterial growth in different protein formulations



The formulated media consisting of protein source and agar supported the growth of all test organisms (Fig. 1). Among the four alternative protein sources, *Klebsiella* sp. showed less growth in most of the protein formulations. Although the growth of other organisms did not differ significantly (P value > 0.05), *Klebsiella* sp. grows better than *Bacillus* sp. in black gram. It is interesting to note that *Staphyllococcus* sp. grows better than the other test organisms on nutrient agar and grows comparatively slowly in all other protein formulations (as far as time is time duration for initial growth is concerned). The overall growth of *Klebsiella* sp. was observed to be less in all growth formulations tested except for black gram.

When each of the test organisms was grown in various protein formulations the following results were obtained. All the test organisms showed significant growth in all the protein sources but comparatively less growth was observed in green gram. However similar less growth was observed in black gram as well for bacteria such as *Bacillus* sp. and *Pseudomonas* sp. All the test organisms show best growth in nutrient agar and among the protein formulations, soya meat is the best alternative except for *Klebsiella* sp.

A number of studies have been carried out to find alternative source of culture media to replace nutrient agar. In a study Sago was effectively used to replace nutrient source as well as agar for the growth of selected bacteria [3]. In another study vegetable was used as a nutrient source with agar for microbial growth. Annan-Prah et al., has used Cowpea as a nutrient source with agar for the growth of selected bacteria [1].

Nutrient agar 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 litre of nutrient agar medium whereas it costs less than 1 LKR to prepare different protein formulations. Thus the use of different protein formulations as culture media in laboratories with basic facilities is very much feasible and cheaper when compared to commercially prepared nutrient agar. Although these protein formulations can be prepared instantly, they can even be stored for more than a month at room temperature in tropical climate.

CONCLUSION

Based on the findings of this study, it is concluded that, *Staphyllococcus* sp. generally grows well in all the protein formulations and *Klebsiella* sp. generally grows least in the protein formulations tested. Among the different media used, all the tested bacteria showed higher growth in nutrient agar. However, Soya meat agar (soya meat + agar) is an effective alternative culture media source next to nutrient agar to grow bacteria as shown by the number of colony growth. All the tested organisms showed the least growth in green gram.

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 composition of the protein formulations and biochemical characteristics and morphology of the bacteria in different protein formulations and more test organisms (bacteria) should be used to identify the suitability

of using these protein formulations as an alternative general purpose medium. It is recommended that further research be conducted with other possible protein sources for the production of bacterial culture medium.

Acknowledgement
We thank Mrs Badrinath Supathini for her timely help with statistical analysis.

REFERENCES

[1] Annan-Prah A., Akorli S.Y. and Sedofia K.B. *African journal of Microbiology Research* (**2010**). , Vol.4 (23):2626-2628.
[2] S. Tharmila, E. C.Jeyaseelan and A. C. Thavaranjit. *Archives of Applied Science Research* (**2011**). Vol.3 (3):389-393.
[3] R. Kapilan and A. C. Thavaranjit. (**2008**). *Journal of Science of the University of Kelaniya,* Vol.4:11-14.
[4] M.Deivanayaki and Antony Iruthayaraj. (**2012**) *International Journal of Biosciences (*IJB) 2012 Vol. 2 No. 5 pp. 47-51.