



Utilization of Dry Baby Shrimp Powder as an Alternative to Peptone in Culture Media.

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ABSTRACT –

*Peptone is most widely used key ingredient of bacteriological culture media for cultivation of microorganisms. This is protein hydrolysates, comprised of polypeptides, peptides, amino acids, inorganic salts and thus provides microorganisms mainly nitrogen and minerals. Despite its advantages, high cost of peptone restricts its uses in school and college laboratories for growing microorganisms. This scenario demands an economical as well as equally or more efficient alternative to peptone for preparation of culture media. The main objective of the present study was to find out alternative source of nitrogen in culture media. The powder of waste part of dry baby shrimps was used as an alternative to peptone in culture media. The efficiency of this alternative source was assessed by studying growth pattern of three test organisms *Bacillus* spp., *Shigella* spp. and *Staphylococcus* spp. on agar and in broth formulations of culture media with powder of dry baby shrimps in comparison with culture media containing peptone and without peptone. Both agar and broth formulations of culture media with dry baby shrimps powder showed significant growth ($P < 0.05$) of test organisms. Also, dry baby shrimps as an alternative to peptone was found to be economically viable as it is readily available in local market at low cost. Thus, baby shrimp powder was found to be economic and efficient alternative to peptone for preparation of bacteriological culture media.*

Keywords: *Peptone alternative, Dry baby shrimp, Culture media, Nitrogen source, Microorganisms*

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INTRODUCTION

The microbial culture media are used for cultivation of microorganisms in laboratories. The various ingredients are used to prepare microbial culture media that support microbial growth. Because of wide nutritional requirements of microorganisms, various media with appropriate nutrients have been designed for cultivation of different kind of microorganisms(1). The major components of culture media include carbon and nitrogen sources along with minerals and vitamins in adequate quantity. However, the composition of media may vary as per their uses.

The nitrogen can be supplied as in the various forms like amino acids, urea, ammonia and other inorganic nitrogen compounds. Peptone is basic ingredient of most of culture media that serves as source of nitrogen, minerals and growth factors to microorganisms and also acts as buffer in media. It is protein hydrolysates containing peptides, amino acids, inorganic salts, carbohydrates and vitamins(2). It is water soluble and doesn't undergo coagulation upon heating. Thus, peptone is the most widely nitrogen source used in culture media as it supports growth of microorganisms excellently(3). The ready made peptones are available commercially for preparation of culture media in laboratories. However, these peptones are too expensive for laboratories with limited resources to employ. The cost of peptone of Himedia (RM001-500g) is Rs.1993. Therefore, the cost of peptone is found to be a major barrier for its uses in school and college laboratories. This scenario demands for finding cheaper alternatives to peptone for preparation of culture media. Dry baby shrimps are excellent source of protein with well-balanced amino acids and significantly high amounts of other nutrients including micronutrients such as calcium and selenium(4). It is readily available at low cost in local market. Since, dry baby shrimps could be a potent source of nitrogen to microorganisms and thus, peptone can be replaced with powder of dry baby shrimps in culture media for cultivation of microorganisms in laboratories. Thus, the present study focused on use of dry baby shrimp powder as a cheaper alternative to peptone in culture media.

MATERIAL AND METHODS

Collection of dry baby shrimps

Dry baby shrimps were collected in sterile polythene bags from Satara fish market and stored at dry place and at low temperature till use.

Preparation of dry baby shrimp powder

The collected baby shrimps were washed with distilled water until the excess salt was removed from them and were dried in sunlight. After proper drying, they were finely powdered using electric blender and sieved to get fine powder. The powder was kept in dry container until its use.

Formulation of solid and broth culture media with dry baby shrimp powder:

The peptone in nutrient medium was replaced by 1 % of fine powder of dry baby shrimps (w/v) to prepare agar and broth media while all other components of nutrient media were added as per regular composition. All agar and broth media were autoclaved at 121°C for 15 minutes.

Microbial Inoculation for study of growth profile

Overnight incubated cultures of test organisms namely *Bacillus spp*, *Shigella spp* and *Staphylococcus spp* were used to prepare suspensions and standardized its density by adjusting O.D. at 520 nm by serial dilution technique. Then 0.1 ml of suspensions of each of test organisms were inoculated on the surface of sterile formulated agar media by four quadrant streaking technique.

The same cultures of test organisms were inoculated in 250 ml Erlenmeyer flask containing sterile 100 ml broth media with 1% dry baby shrimp powder and incubated at 37°C on shaker incubator for 24 hrs. After incubation, bacterial growth of each test organism was measured in term of turbidity by using spectrophotometer (optical density at 520 nm).

The same cultures of test organisms were serially diluted and used to determine standard plate count (SPC) of test organisms on same formulated agar media (5).

Same procedure was repeated to measure growth of test organisms on nutrient media with 1% peptone (w/v) and without peptone. Bacterial growth profile on formulated culture media with 1% dry baby shrimp powder was compared with bacterial growth pattern on nutrient media with 1% peptone (w/v) and without peptone.

Statistical Analysis

All the experiments were performed in triplicate. All data obtained from above experiments were expressed as mean and standard deviation. The differences in efficiency of formulated media with 1% dry baby shrimp powder and nutrient media with 1% peptone (w/v) and without peptone were analysed by Analysis of Variance (ANOVA) ($P < 0.05$) followed by RBD by using R software (4.1.2).

RESULT

It was observed that formulated agar media with 1% dry baby shrimp powder (w/v) supported growth of test organisms. Fig. 1 showed excellent growth of all test organisms namely *Bacillus spp*, *Shigella spp*, *Staphylococcus spp* on formulated agar media (with 1% of dry baby shrimp powder) and on regular nutrient agar media (1 % peptone) on the other hand, a very little growth was observed on nutrient media without peptone. This result suggested that formulated agar medium and regular nutrient agar medium is equally efficient in promoting bacterial growth.

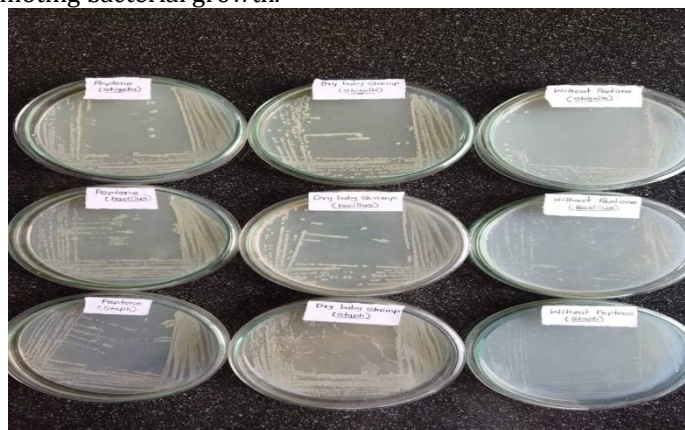


Fig. 1 Growth of test organisms namely *Bacillus spp*, *Shigella spp*, *Staphylococcus spp* on formulated agar medium with 1% dry baby shrimp powder (w/v), Nutrient agar with 1% Peptone (w/v) and without peptone obtained by four quadrant streaking method.

The standard plate count of *Bacillus spp* on formulated media with 1% dry baby shrimp powder(w/v) and nutrient agar media with 1 % peptone(w/v) were found to be 200.8CFU/ml and 193.4CFU/ml respectively while on nutrient media without peptone,it wasfound only 20.6 CFU/ml. For *Staphylococcus spp*, SPC on formulated media with dry baby shrimp powder was 286.8CFU/ml and nutrient agar media with peptone and without peptone were 257 CFU/ml and 33.2 CFU/ml respectively.For *Shigella spp*, SPC on formulated medium with dry baby shrimp powder and nutrient agar medium with peptone were 195CFU/ml and185CFU/ml respectively while on medium without peptone it was 22.2CFU/ml (Table 1). SPC of all test organisms on formulated media with dry baby shrimp powder were found to be slightly higher thanSPC of same test organisms on nutrient media with 1 % peptone. Thus,formulated medium with 1% dry baby shrimp powder was found to beefficient to promote the growth of test organisms(Fig 3).

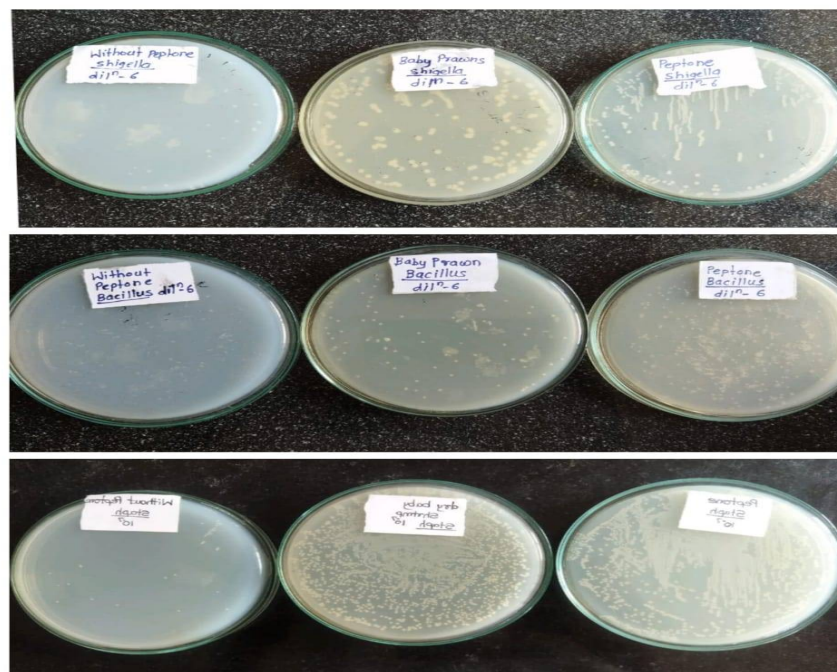


Fig. 2 Standard plate count of test organisms namely *Bacillus spp*, *Shigella spp*, *Staphylococcus spp* on formulated agar medium with 1 % dry baby shrimp powder(w/v), nutrient agar with 1 % Peptone(w/v) and without peptone.

Table1.Standard platecount of test organisms on formulated media with 1 % dry baby shrimp powder(w/v),nutrient agar with 1% peptone(w/v) and without peptone.

Test organisms	Dilution	Standard Plate Count (SPC /ml) on media		
		1% Dry baby shrimp powder(w/v)	Without peptone	1 % Peptone(w/v)
<i>Staphylococcus spp</i>	10 ⁻⁴	286.8	33.2	257
<i>Bacillus spp</i>	10 ⁻⁴	200.8	20.6	193.4
<i>Shigella spp</i>	10 ⁻⁴	195	22.2	185

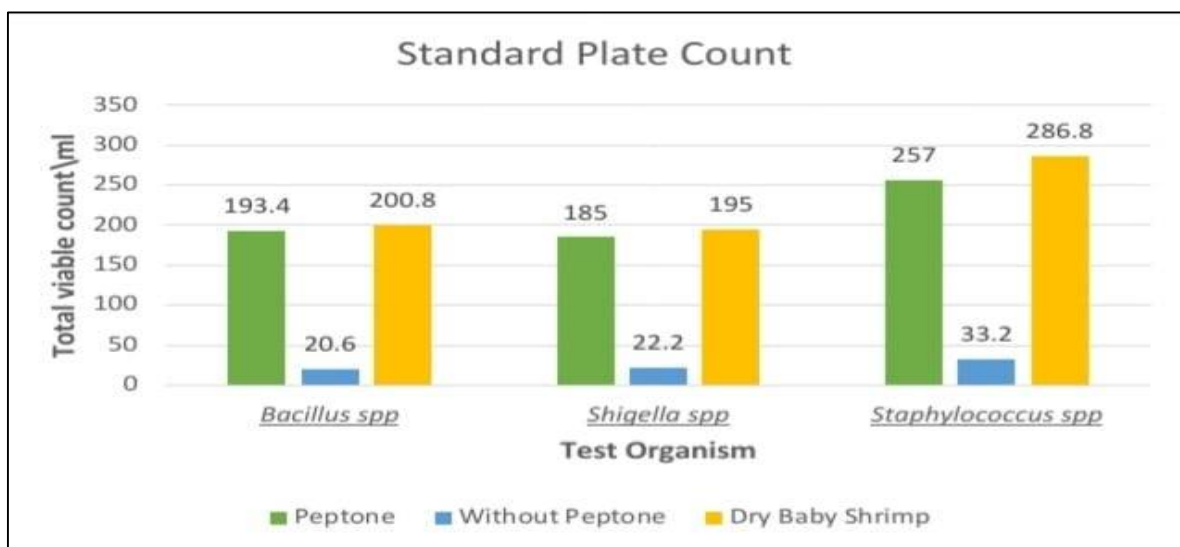


Fig.3 Standard plate count of test organisms on formulated media with 1% dry baby shrimp powder(w/v), Nutrient agar with 1% Peptone and Without peptone.

The growth of all three test organisms namely *Bacillus spp*, *Shigella spp*, *Staphylococcus spp* in different broth media were determined in form of cell density measured at 520 nm. In Table 2, it was observed that growth of all three test organisms in formulated media with 1% dry baby shrimp powder(w/v) is slightly higher than that of all test organisms in nutrient broth with 1% peptone while very little growth of test organisms was observed in nutrient broth without peptone.

Table 2. Growth of test organisms measured in term turbidity of test organisms.

Test organisms	Optical Density at 520 nm		
	1% Peptone(v/w)	1% Dry baby shrimp powder(w/v)	Without peptone
<i>Staphylococcus spp</i>	0.606	0.582	0.042
<i>Bacillus spp</i>	0.456	0.580	0.040
<i>Shigella spp</i>	0.454	0.502	0.076

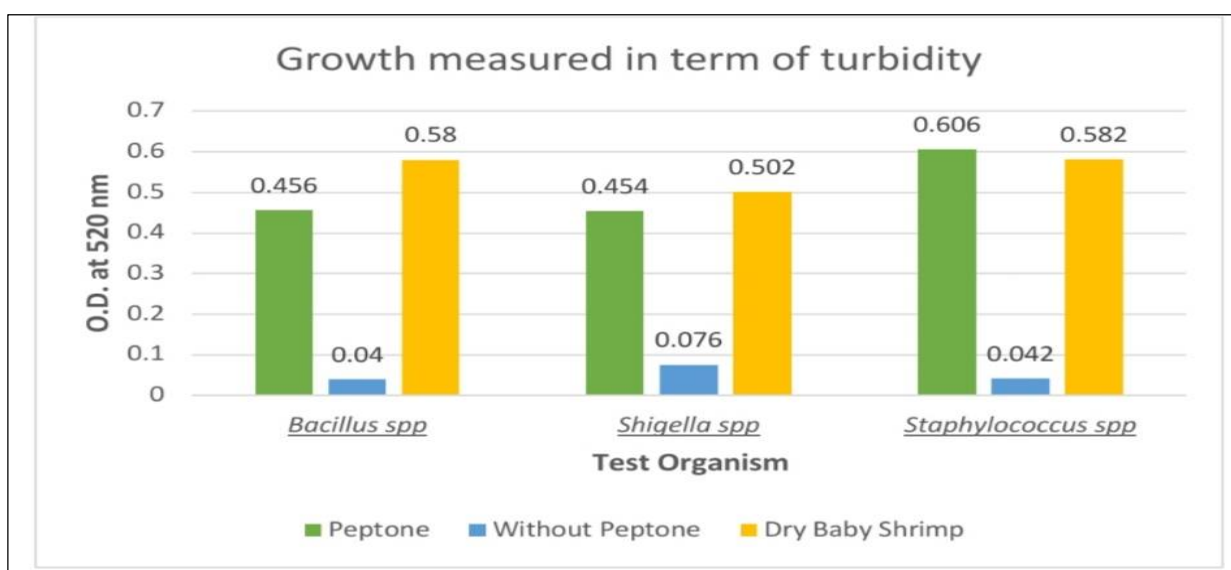


Fig.4 Growth of test organisms measured in the term of turbidity at 520 nm.

Statistical Analysis:

The data obtained by measuring standard plate count on agar and growth in broth media of test organisms namely *Bacillus spp*, *Shigella spp* and *Staphylococcus spp* were analysed by ANOVA [(P<0.05) followed by RBD by using R software (4.1.2)]. Both agar and broth formulations of culture media with 1% dry baby shrimp powder(w/v) showed significant growth (P<0.05) of test organisms in comparison with nutrient media with 1% peptone and without peptone.

CONCLUSION

The overall results showed that culture media with 1 % dry baby shrimp powder supported the growth of test organisms namely *Bacillus spp*, *Shigella spp* and *Staphylococcus spp*. Moreover, statistical analysis revealed that formulated medium with 1% dry baby shrimp powder(w/v) was found to be more efficient (P<0.05) to promote growth of test organisms than nutrient medium. In addition, dry baby shrimps are readily available in local market at low price. Thus, easy availability, low cost and equal or more efficiency make dry baby shrimp powder potential cheaper alternative to peptone in microbial culture media.

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