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SAGO AS A MEDIUM FOR "IN VITRO" CULTURE OF SOME COMMON SOIL BACTERIA

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ABSTRACT

Nutrient agar (NA) medium is universally used as a general purpose medium for the culture of a broad range of bacteria and the cost of this commercially available medium is considerably high. Sago (*Metraxylon sagu*) contains a considerable amount of starch and a small amount of reducing sugars and is not used as a staple food in Sri Lanka. This study was aimed to find out the suitable composition of the sago medium compared with NA and to carry out growth studies of soil bacteria on sago medium. Media were prepared in different compositions by addition of different amount of sago in 100mL volume (25mL of distilled water + 75mL of young king coconut sap) separately. Bacterial suspensions (5.21×10^6 cfu/ml, SD=1.12) of *Bacillus*, *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Serratia* and *Staphylococcus* were transferred on the selected sago medium separately by using spread plate techniques. There was no significant difference between sago and NA media in number of the colonies (t test; p = 0.05). Colonies appeared earlier in NA media than in sago media. But the colony morphological characters such as shape/form, margin, elevation, colour and size were almost similar to all bacteria selected on both media, except consistency. Thus, instead of NA the low cost and easily available sago medium could be recommended for the cultivation of soil bacteria.

Key words - Sago medium, Nutrient agar, solidification time, *Metraxylon sagu*

INTRODUCTION

Nutrient agar (NA) medium is universally used as a general purpose medium for the cultivation of broad range of bacteria (Brook *et al*., 1998). Cost of this commercially available medium is fairly high (500g bottle – SLR 12,000/=). Therefore there is a necessity to formulate new media with easily available low cost substances for substituting NA medium. Sago (*Metraxylon sagu*) contains considerable amount of starch and small amount of reducing sugars and it is not much used as a staple food in Sri Lanka. It is easily available in the local market at reasonable prices (1Kg – SLR 80/=). In addition to the solidification property of sago it may help in media preparation too. Young king coconut is also commonly available with enough nutrients and can be selected to prepare this medium. This study was aimed to find out the suitable composition of the sago medium compared with NA and to carry out the growth studies of soil bacteria on sago medium and to test the effect of the medium for bacterial growth.

MATERIALS AND METHODS

Sago, king coconut water (endosperm liquid) and distilled water were used for the preparation of the medium. Media were prepared in different compositions by the addition of different weights (gm) of ground sago such as 1,2,3,4,5,6,7 and 8 grams in 100ml volume (25 ml of distilled water + 75 ml of king coconut water) separately. Sago was dissolved by using a magnetic stirrer on a hot plate. Dissolved media were sterilized by an autoclave at 121°C for 20 minutes under the pressure of 15lbs/inch^2 and were poured into sterile petridishes separately. Above procedure was carried out in triplicate. For each concentrations Nutrient agar (NA) plates were used as control. Media solidification time was noted for each concentration. The same procedure was repeated 8 times and the results were analyzed statistically. The medium with the most suitable composition for solidification was selected and used for the bacterial growth studies.

Bacterial suspensions (5.21×10^6 cfu/ml, SD = 1.12) of *Bacillus* sp, *Pseudomonas* sp, *Klebsiella* sp, *Enterobacter* sp, *Serratia* sp and *Staphylococcus* sp were transferred on the selected sago medium separately by using spread plate techniques, with 8 replicates. They were also inoculated on to Nutrient agar (NA) as controls. After 2 days of incubation at room temperature, number of colonies was counted and colony morphology (shape/form, margin, elevation, colour, size, consistency) was studied on both media. The results were analyzed statistically.

RESULTS

Statistical analysis (t test, p= 0.05) showed, that there was no significant difference between the setting time of NA and sago medium of the composition of 6 gram sago + 75 ml king coconut water and 25ml distilled water (Table 1). Therefore, this composition was more suitable in the preparation of sago medium.

Table 1: Mean solidification time (min) of the different concentrations of sago media (SE = 1.04)

	Composition of the media			Mean solidification time (min)
	Sago (gm)	Dist.water (ml)	King coconut water (ml)	
Sago medium	01	25	75	Did not set
	02	25	75	>45
	03	25	75	35
	04	25	75	28
	05	25	75	21
	06	25	75	16
	07	25	75	10
	08	25	75	05
NA medium				17

Table 2: Mean number of bacterial colonies on Sago and NA media (SD = 5.31)

Bacterial genera	No.of bacterial colonies ($x10^6$ cfu/ ml)	
	Sago medium (6%)	Nutrient agar
Bacillus	6.35	7.02
Pseudomonas	8.10	9.04
Klebsiella	10.03	12.01
Enterobacter	4.70	6.80
Serratia	6.71	7.10
Staphylococcus	7.04	10.11

All the tested bacterial genera showed, growth on 6% sago medium (Table 2). Composition of the sago medium consisting of six grams of sago, 75mL of king coconut sap and 25mL of distilled water was selected. There was no significant difference between sago and NA media in number of colonies (t test, p=0.05). Colonies appeared earlier on NA media rather than sago media. But in colony morphology except consistency other characters such as shape/form, margin, elevation, colour and size were almost similar to all bacteria selected on both media.

Sago medium remained unchanged for nearly one week time but that for the NA was more than one week. Incubation period was one and half day longer in sago medium than NA medium. Chances of contamination in sago medium were also low when compared with NA medium.

CONCLUSIONS

Six grams of sago, 25 mL of distilled water and 75 mL of king coconut sap water were the most suitable composition in preparation of the sago medium for bacteria. Thus, instead of NA low cost and easily available Sago medium could be recommended for the culturing of soil bacteria such as *Bacillus*, *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Serratia* and *Staphylococcus*. Further studies should be done to test the effect of sago media for the growth of other soil bacteria and bacteria from various habitats.

REFERENCES

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