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The Suitability of a Various Gelling Agents as Agar Substitute in Microbiological Growth Media

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Abstract: ***Three gelling agents were investigated as agar substitutes. These include cassava starch, Potato starch and carrageenan. Among these carrageenan was found to be a alternate candidate for agar. Media solidified with 3% carragenan was more transparent and supportive for the growth of seven test bacteria (E.coli, Klebsiella, Proteus, Pseudomonas Staphylococcus aureus, Salmonella Typhi and Serratia) as good as agar. Comparing carrageenan to agar, it also performed better in terms of cost-benefit analysis. Carrageenan concentration varies in various cultural media compared to agar.***

Keywords: Agar, Gelling agents, Carrageenan

1. Introduction

The establishment of culture media allowed the development of microbiology in the nineteenth century. Bacterial culture was the first method developed to study the human microbiota , using an artificial medium that allows growth and isolation of bacteria. The first to have cultured a bacterium in a reproducible way was Louis Pasteur in 1860 thanks to the development of the first so-called artificial culture medium [1]. A culture medium may either be a liquid or gelled substance that supports the growth of microorganisms under laboratory conditions [2]. A growth medium or culture medium is a solid, liquid or semi solid designed to support the growth of a population of microorganisms or cells via the process of cell proliferation. Solidifying and gelling agents are Some microbial and plant origin proteins and colloidal polysaccharides act as solidifiers or stabilizers for solid or semi solid media preparation .Culture media contain all the elements, the most bacteria need for growth and are not selective, so they used for the general cultivation and maintenance of bacteria kept in laboratory culture collections. The culture media contains peptones, nutrients, meat, malt, yeast extracts, buffering agents, pH indicators, solidifying agents and selective agents [3]. The culture medium to commonly used solidifying agent is agar. Agar is an inert polysaccharide obtained from sea weeds. Concentration of agar in solid media is 1.5-2% and in semisolid media 0.3-0.5%. Agar may contain some impurities including inorganic salt, a small amount of protein, long chain fatty acids. The mineral present in agar are mg2+, ca2+. It has high setting strength. Melting temperature is 90-95 $^{\circ}c$. Setting temperature is 40-45$^{\circ}c$. Agar is available as powder form and strand form. Two types of agar are present- New -Zealand agar and Japanese agar. [4]. The other solidifying agents are Silica gel, Agarose, Pectin, Gelatin, Carrageenan calcium, Cassava starch, Pluronic Polyol F127, Arrow root powder, Corn flour, Gel rite, Coconut powder, Rice powder, katira powder, Isubgol husk, and Phytagel. Essentially like agar, cassava starch is an acidic polysaccharide (Davis et al 1973) consisting in its powdered form of 77% carbohydrate, 21% lipid and 2% protein. This study attempts to explore the possibility of using cassava starch as a solidifying agent. Carrageenan: It is extracted from the cell wall of marine algae Chondrus crispus. It is also used as agar replacer due to gelling ability (especially K salt of carrageenan). It has limitations for the growth of several marine bacteria [1]

2. Materials and Methods

The experimental research carried out in our institution's undergraduate laboratory.

2.1 Gelling agents and source

The gelling ability of various gelling agents was examined with Nutrient agar, Macconey agar, Blood agar, and Muller Hinton agar as the basal medium. Treatments consisted of Potato starch, cassava starch and Carrageenan. The solidifying agents were bought on the nearby market. In these studies, different gelling substances were used to substitute of the agar-containing cultural medium. Seven bacteria were examined in agar substituted media: E. coli, Klebsiella spp, Proteus spp, Salmonella Typhi, Pesudomonas spp, Staphylococcus aureus, and Serretia. Colonies were then examined in various gelling agents containing medias and cost benefit ratio.

2.2 Cost benefit ratio

Cost benefit ratio of various gelling agents was also calculated by comparing their price with the standard price of agar.

3. Result and Discussion

Carrageenan at 3% was the only agar substitute evaluated that produced promising outcomes when compared to agar. The other agents either made lumps right after autoclaving and well before media was poured into Petri plates because

they were runny or were so thick(Table 1). On the other hand, plates toughened with Carrageenan were absolutely transparent and rock solid. In comparison to agar, the bacteria used in this research provide satisfactory cultural characteristics.

Table 1: Gelling Behavior of Various Gelling agents Compared with Agar

Medium (agar substituted compounds)	concentration	Observation
Nutrient Potato starch agar	10%	Very thick consistency.
	5%	Thick consistency.
Nutrient Cassava starch agar	10%	Thick consistency.
	5%	Not too thick, poured in plates but not set.
Nutrient Carrageenan agar	3%	Solidified better, Less contamination, and medium is more clear from the nutrient agar plates.
Blood carrageenan	3%	Poured in plates but not set.
MacConkey carrageenan	3%	Too thick consistency.
	1.5%	Solidified.
Muller Hinton carrageenan	1.5%	The medium is precipitated in bottom.

4. Conclusion

Carrageenan solidifies as well as agar in solidifying substances other than agar. There is greater transparency than in the agar-containing media A drawback of carrageenan in media processing is the variation in carrageenan concentration in various media.

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