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ASSESSMENT OF GELLING AGENTS FOR THE FORMULATION OF CULTURE MEDIA

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ABSTRACT

Two gelling agents were investigated as agar substitutes. These are cassava starch and guar gum (Cymopsis tetragonolobus). Among these two, guar gum was found a promising alternate candidate for agar. Media solidified with1.2% guar gum was transparent and supportive for the growth of microorganism. Agar has $1.2x10^{10}$ and $3.45x10^{10}$ colony count for Aspergillus niger and E. coli respectively while guar gum has $9.0x10^{9}$ and $3.45x10^{10}$ colony count. Guar gum also excelled in terms of cost benefit ratio when compared with agar. Guar gum fortified media was found to cost N6,500 per liters as compared to agar supplemented media costing N19,500 per liter. Further, guar gum is easily available and can be added with ease thereby serving as a suitable and inexpensive substitute of agar and thus, can be adopted for routine microbiological testing in resources poor countries.

Keywords: Guar gum, Media, Agar, Gelling agents

INTRODUCTION

Gelling agents or thickening agents are substances that can increase the viscosity of a liquid without substantially changing its other property. Typical gelling agents include natural gum, starches, pectin, agar and gelatin. Often they are based on polysaccharides or proteins. For tissue culture, agar represents one

of the most expensive and commonly used media components contributing about 70% of total production cost (Ullah *et al*., 2015).

Gelling agents are required for formulating both solid and semisolid media, vital for the isolation of microorganism. Gelatin was the first gelling agent to be discovered but it soon paved the way for agar, which has far superior material qualities. Sources depletion, issues with polymerase – chain- reaction and inability to sustain extremophiles e.t.c., necessitate the need of other gelling agents. (Das *et al.,* 2015).

Gelling agent are added to culture media to increase its viscosity, gelling agent provide firmness to the medium and influence its diffusion characteristics. Diffusion rate is the dependent on the viscosity of the medium, which subsequently depend on the concentration and physicochemical characteristics of the agents (Das *et al*., 2015).

Food grade agar has also been reported as a low cost alternative in the preparation of solid microbiological medium. The food grade agar is comparable to bacteriological agar in terms of its gelling and stability properties (Petrovski and Tillet, 2012).

Media for microbial culture can be classified as liquid or gelled substances that support the growth of microorganisms under laboratory conditions. Various media are used for growing different types of organisms which include those used for cell culture, derived from plant or animal and other microbiological culture media used for growing microorganisms (Mateen *et al*., 2012).

METHODOLOGY

Sample Collection

Potato, corn, sodium chloride and cassava starch were purchased at Gwallameji market in Bauchi, Nigeria. While dextrose, peptone, beef extract, yeast extract, guargum and xanthan gum were purchased from Jos, Plateau state.

PreparationofCassavaStarch

Tubers of cassava obtained were peeled, washed and grated. A blender was used to blend 1kg of the grated tuber into paste. The paste was then strained into a clean plastic bucket using a cheese cloth and the solution obtained was topped up with 1 liter of distilled water. The starch solution was left in the laboratory

for 24hours and the supernatant was poured off to obtain a clean starch paste (Kwoseh *etal*.,2012)

Pre-treatment of Sample

The cassava starch were crushed and allowed to dry in an hot air oven at 160^{o}C for 1 hour, it was then sieved using 0.006mm sieve. The corn was ground into powder using an electric grinder and sieved using 0.006mm.

Media Formulation

Preparation of Cornmeal Agar

The cornmeal medium was prepared by combining 3g of cornmeal powder and 0.9g of agar, the mixture was dissolved in 60ml of distilled water and was gently heated with intermitted stirring to dissolve. The media was then sterilized by autoclaving at 121^{o}C for 15 minutes and allowed to cool to 45^{o}C before dispensing into sterile petri dishes in 20ml volume.

Preparation of Cornmeal Cassava Starch Medium

The medium was prepared by combining 3g of cornmeal powder and 7.2g of cassava starch, the mixture was dissolve in 60 ml of distilled water in a 250ml conical flask and was gently heated with intermitted stirring to dissolve. The medium was sterilize by autoclaving at 121^{o}C for 15 minutes and allowed to cool to 45^{0}C before dispensing into sterile petri dish in 20ml volume.

Preparation of Cornmeal Guar GumMedium

The cornmeal guargum medium was prepared by combining 3g of cornmeal powder and 0.72g of guar gum powder, the mixture was dissolve in 60 ml of distilled water, gently heated with intermitted stirring to dissolve. The medium was sterilized by autoclaving at121^{0}C for 15 minutes and allowed to cool to 45^{0}C before dispensing into sterile petri dishes.

Preparation of Potato Dextrose Medium Agar

Potato infusion was prepared by washing the potato with distill water, then 12g of sliced unpeeled potato was boiled in 60mls of distilled water for 30 minutes, it was filtered through cheesecloth, it was mixed with 1.2g of dextrose, 1.2g of agar and water and boiled to dissolve, it was sterilized by autoclaving at 121^{o}C

for 15 minutes and was allowed to cool before dispensing into sterile petri dishes.

Preparation of Potato Dextrose Cassava Starch Medium

PotatoDextrose Cassava starch Medium was prepared by boiling 12g of sliced unpeeled potatoes in 60 ml of distilled water for 30 minutes, it was filtered through a cheese cloth, mixed with 1.2g of dextrose and 7.2g of cassava starch, boiled to dissolve and was sterilized in an autoclave at 121^{o}C for 15 minutes, allowed to cool and dispensed into sterile petri dishes.

Preparation of Potato Dextrose Guargum Medium

Potato Dextrose Guar gum Medium was prepared by boiling 12g of sliced unpeeled potatoes in 60ml of distilled water for 30 minutes, it was filtered through a cheesecloth, mixed with 1.2g of dextrose and 0.72g of guar gum, boiled to dissolve, sterilized by autoclaving at 121^{0}C for 15 minutes, allowed to cool at 45^{0}C and dispensed into sterile petri dishes in 20 ml volume.

Preparation of Nutrient Medium Agar

Nutrient Medium was prepared by combining 0.9g of agar, 0.3g of peptone, 0.18g of beef extract and 0.3g of sodium chloride. A mass of 1.68g was dissolve in 60ml of distilled water,heated with intermitted stirring to dissolve. The medium was sterilize by autoclaving at 121^{0}C for 15 minutes and allowed to cool to 45^{0}C and dispensed into sterile petri dish in 20ml volume.

Preparation of Nutrient Cassava Starch Medium

Nutrient Cassava starch Medium was prepared by combining 7.2g of cassava starch, 0.3g of peptone, 0.18g of beef extract and 0.3g of sodium chloride. The mixture was dissolve in 60 ml of distilled water, heated with intermitted stirring to dissolve. The medium was sterilize by autoclaving at 121^{0}C for 15 minutes, allowed to cool and dispensed into petri dishes.

Preparation of Nutrient Guar gum Medium

Nutrient Guar gum Medium was prepared by combining 0.72g of guargum powder, 0.3g of peptone, 0.18g of beef extract and 0.3g of sodium chloride. The mixture was dissolve in 60ml of distilled water, heated with intermitted stirring

to dissolve. The medium was sterilize by autoclaving at 121^0C for 15 minutes, allowed to cool to 45^0C and dispensed into sterile petri dishes.

Testing of Gelling Ability

The gelling strength was measured by using a tripod stand with a central rod that is used to impart pressure on the agar. The lower end of the rod has a spherical portion, which rest on the medium surface. The upper end of the rod has a platform on which standard weighed are placed. The spherical portion of the central rod was placed on the medium and weight were placed on the upper platform one by one and observed for some time until the agar breaks. While calculating the gel strength, the weight of the central rod was deducted. The force imparted by the rod on the agar surface was calculated by the formular: WPr^2 (Basu,*etal*., 2005)

Where, W = weight kept on the platform

r = radius of the spherical portion at the lower end of the central rod.

P= 3.14

Growth Enhancing Ability

Preparation of Inoculum

Mc Farland's turbidity standard was prepared by adding 0.6ml of 1% (v/v) Barium chloride ($Bacl_2$) to 99.4ml of 1% sulphuric acid (H_2SO_4). The absorbance of the preparation was measured using a spectrophotometer with a 1cm light path set at 600 nm (nanometer), the equivalent concentration of *E.coli*in 0.5 Mc Farland turbidity standard is 1.5 x 10^8 colony forming units (CFU/ml). A small volume of the turbid solution was transferred to a capped tube and another similar tube was used to prepare a mixture of actively growing fungi to achieve the same turbidity as the standard. A volume of 0.1ml of the inoculum was poured on the surface of the medium and was spread over the surface of agar using a sterile bent glass rod and was then incubated at 37^0C for 48 hours. The developing colonies were observed (Udosen *etal*., 2017).

Microbial Count/Comparism with Standard

The numbers of colonies on each formulated medium containing various gelling agents were counted using a colony counter and compared with media containing agar.

$$A + B + C = \frac{X}{3}$$

Statistical Analysis

One-way ANOVA analysis was used in comparing results obtained.

RESULTS

Concentration of Gelling Agent used for Solidification.

Table 1 shows the various concentration of gelling agents that were added to the basal medium

Gelling Ability

Table 2 shows the gelling ability of various gelling agents compared with agar

Growth Enhancing Ability

Table 3 shows the growth enhancing ability of various gelling agent

Cost Benefit Ratio

Table 4 shows the cost benefit ratio of various gelling agents as compared with the standard price of agar.

Table 1 Concentration of various gelling agents used for solidification of media

Gelling Agents	Concentration %					
Cassava Starch	2	3	3.5	4	4.5	5
Guar Gum	0.5	1	1.2	1.3	2	1
Agar	0.5	1	1.5	-	-	-

Table 2. Gelling Ability of various gelling agents compared with agar

Gelling Agents	**Gelling Ability (Dynes/cm^3)**
Cassava Starch	282.6
Guar gum	339.2
Agar	395.64

Total count of test organism on cassava starch, guar gum and agar

Table 3: Growth Enhancing Ability

Organism	Medium	Colony Count (CFU/ml)
Aspergillus	Cornmeal Agar	$1.2x10^{10}$
	Cornmeal Guar Gum	9.0×10^{9}
	Cornmeal Cassava Starch	$4.5x\ 10^{8}$
	Potato dextrose Agar	$1.05x10^{11}$
	Potato Dextrose Guar Gum	$9.0x\ 10^{9}$
	Potato dextrose cassava starch	$4.5x10^{8}$
E. coli	Nutrient Agar	$3.45x10^{10}$
	Nutrient guar gum	$3.45x10^{10}$
	Nutrient cassava	$6.75x10^{7}$

Table 4. Cost Benefit Ratio of Agar, Guar Gum and Cassava Starch

Gelling Agents	Cost/ 500(N)g
Agar	19,500
Guar Gum	6500
Cassava Starch	500

DISCUSSION

Cassava starch gelled medium had poor clarity compared to agar. Clarity of the cassava starch gelled medium was satisfactory improved with addition of 0.3 % of agar and it also improved gel strength. Plate fortified with guar gum, on the other hand, were rock solid with clear consistency. Guar gum at 1.2% was the only gelling agent that gave promising result when compared to agar. (Table 1) Guar gum is obtained from guar bean or cluster bean (*Cyamopsis tetragomoloba*) which is an annual legume grown in Pakistan, India, Australia, china, USA and Africa. It grows best when there is frequent rainfall, but can tolerate acid conditions. The guar seed are de-husked, milled and typically produced as a free flowing, pale off-white colored, coarse to fine ground powder. Guar gum has 85% water-soluble fraction or guaran. It is a nontoxic colloidal polysacharide composed of straight chain mannan, with a galactose residue attached to every second mannose molecule. Being completely soluble in cold as well as hot water, it hydrates easily to produce solutions possessing very high viscosity at low concentrations. It is economical since it has almost

eight times the water-thickening potency of corn starch. Further, only a minimal amount is required for obtaining sufficient viscosity.

Guar gum has been used previously as a substitute of agar, mainly for plant tissue culture media. The present studies however, focused on its role as a substitute of agar in microbiological culture media. After agar, guar gum has the highest gelling ability (Table 2).

Number of fungi colonies (*Aspergillus*) and bacteria colonies (*E. coli*) on guar gum, cassava starch and agar where obtain (Table 3). Guar gum excelled after agar. It beneficial influence on the growth of fungi could have been due to the presence of a colloidal polysaccharide which is composed of straight chain mannan and galactose residue attached to every second mannose molecule.

The properties of guar gum including its polysachariotic and colloidal nature, resistance to enzymatic activity, good gelling ability even in cold water, and reasonable clarity in gelled form, are indicative of its potential to become a universal gelling agent in culture media for microbial growth.

The financial constraints in research or countered commonly in developing countries like Nigeria warrant that a cheap alternative be sought. Guar gum, the gelling agent used in the present study, unlike agar absorb water even at room temperature that results in its gel formation. Guar gum being 3 times cheaper than agar could prove to be a cheap alternative (Table 4). It source, C. tetragonoloba, is an easily cultivable plant, and therefore, increase in its demand could be easily met by increasing the area of cultivation.

CONCLUSION

The formulated medium, cornmeal medium, nutrient medium, potato dextrose medium fortified with guar gum has been found to have good gelling ability and supportive for the growth of *aspergillus* and *E. coli.*

RECOMMENDATIONS

1. Further measures should be employed to increase the cultivation of guar seed.
2. Apart from the microorganism used, other organism should be grown (cultured) to ascertain the efficacy of guar gum.
3. Other gelling agent should also be tested if they can be used as agar substitute.

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