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Full Length Research Paper

Suitability of various plant derived gelling agents as agar substitute in microbiological growth media

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Eleven putative gelling agents were investigated as agar substitutes. These included arrowroot (*Maranta arundinaceae*), coconut powder (*Cocos nucifera*), corn flour (*Zea mays var. amylacea*), gel rite (a water-soluble polysaccharide produced by *Sphingomonas elodea*), glue (*Cyanoacrylates*), katira gum (*Cochlospermum religiosum*), guar gum (*Cyamopsis tetragonolobus* L.), isubgol husk (*Plantago ovata*), pectin and rice (*Oryza sativa* L.) powder. Among these, guar gum was found a promising alternate candidate for agar. Media solidified with 2.8% guar gum was transparent and supportive for the growth of three test fungi (*Trichoderma harzianum, Alternaria alternata* and *Alternaria solani*) as good as agar. Guar gum also excelled in terms of cost benefit ratio when compared with agar. Guar gum fortified media was found to cost $ 0.005/L as compared to agar supplemented media costing $ 1.17/L. 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 resource poor countries.

Key words: Guar gum, media, agar, gelling agents.

INTRODUCTION

A culture medium may either be a liquid or gelled substance that supports 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 plants or animals and other microbiological culture media used for growing microorganisms. The use of agar was first proposed by Hesse (1881) for microbiological purpose. By early 1900s, it was widely used as it remains firm at growth temperatures as high as 65°C for many pathogens. Conversely, it melts at approximately 85°C, a different temperature at which it solidifies that is, 32 to 40°C a property known as hysteresis. Moreover, agar is generally resistant to shearing forces but different agars may have different gel strengths or degrees of stiffness (Hesse, 1894; Hitchens and Leikind, 1939). Although, agar is still the most widely used solidifying agent for microbial culture media, the exclusive use of agar may result in over-exploitation of its resources. Moreover, high cost of bacteriological grade agar necessitates that alternatives be sought (Jain et al., 1997; Babbar and Jain, 1998; Jain and Babbar, 2002).

A range of gelling agents such as corn flour (*Zea mays var. amylacea*), isubgol husk (*Plantago ovata*), coconut powder (*Cocos nucifera*) and many others are available; arrowroot (derived from the roots of a tropical South American plant *Maranta arundinacea)* after a fairly complicated process whose end result yields the powdery white arrowroot starch resembling corn flour. Coconut powder is obtained by drying the granulated or shredded white meat of the fully mature coconut kernel, by means of a mechanical air drying. Coconut powder is a free flowing white powder made from pasteurized, homogenized and spray dried natural extract of coconut kernel

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Table 1. Concentrations of various gelling agents used for solidification of media.

Gelling agent	Concentration (%)				
Arrowroot	2	3	4.8	6	7.2
Coconut powder	2	3	4.8	6	7.2
Corn flour	2	3	4.8	6	7.2
Gel rite	2	2.4	2.8	3.2	-
Glue	2	2.4	2.8	3.2	-
Gond katira	2	2.5	3	3.2	-
Guar gum	2	2.4	2.8	3.2	-
Husk	1.2	1.4	1.6	1.8	2.0
Pectin	2	2.4	2.8	3.2	-
Rice powder	2	3	4.8	6	7.2

kernel after the removal of brown testa. Corn flour or cornmeal is flour ground from dried maize or American corn. Gel rite is a water soluble polysaccharide produced by *Sphingomonas elodea*, a bacterium. Glue which is an adhesive, or glue, is a mixture in a liquid or semi-liquid state that adheres or bonds items together. Adhesives may come from either natural or synthetic sources. Katira gum, which has only been available commercially since 1920, forms an extremely strong adhesive with small amounts of water. Katira gum is the resin extracted from a particular tree. It looks like golden orange tan small balls. Katira gum occurs as tears of variable size and of a somewhat crystalline appearance. The tears are translucent, pale yellow, with a slightly acetic odor and a mucilaginous, slightly acetic taste. Guar gum is a polysaccharide with a straight chain of D-mannopyranose units joined by B (1-4) linkages with a side branching unit of a single D-galactopyronase joined to every other mannose unit by L (1-6) linkages. It is a white to yellowish-white powder, practically odorless and has a bland taste. It will disperse and swell almost completely in hot or cold water and is insoluble in organic solvents. The viscosity depends on temperature, time, pH, agitation rate and particle size of the powder. Isubgol husk (Psyllium seed husks) also known as ispaghula, isubgol or psyllium, are portions of the seeds of the plant *P. ovata*, a native of Pakistan (Saglam and Ciftci, 2010). They are soluble in water, expanding and becoming mucilaginous when wet. Psyllium seed husks are indigestible in human beings and are often used as a source of dietary fiber. Pectin was first isolated and described by Henri Braconnot (1825) from Greek-*pektikos*, "congealed, curdled" which is a structural hetero polysaccharide contained in the primary cell walls of terrestrial plants. It is produced commercially as a white to light brown powder, mainly extracted from citrus fruits. Rice powder, also rice flour is a form of flour made from finely milled rice. It is distinct from rice starch, which is usually produced by steeping rice in lye. Rice flour may be made from either white rice or brown rice. To make the flour, the husk of rice or paddy is removed and raw rice is obtained which is then ground to flour.In the recent past, a number of substances namely, agarose (Johansson, 1988), carrageenan (Lines, 1977; Bromke and Furiga, 1991), katira gum (Jain and Babbar, 2002), isubgol (Jain et al., 1997; Babbar and Jain, 1998; Ozel et al., 2008; Atici et al., 2008; Saglam and Ciftci, 2010), kappa-carrageenan (Abbott et al., 1981), starch (Henderson and Kinnersley, 1988; Zimmerman et al., 1995; Nene et al., 1996), guar gum (Babbar et al., 2004), gel rite (Harris, 1985), chickpea dextrose tapioca (CDT) (Nene et al., 1996) and gellan gum (Shungu et al., 1983) have been used. The present studies were undertaken to validate the available data and to look for additional cheap and easily available substitutes of agar to be used in microbiological culture media.

MATERIALS AND METHODS

Gelling agents, source and gelling ability

The gelling performance of various potential gelling agents was investigated with potato dextrose broth as the basal medium. Treatments consisted of arrowroot (*M. arundinaceae)*, coconut powder (*C. nucifera)*, corn flour (*Z. mays var. amylacea)*, gel rite (a water soluble polysaccharide produced by a bacterium *S. elodea*), glue *(Cyanoacrylates)*, katira gum *(Cochlospermum religiosum)*, guar gum *(Cyamopsis tetragonolobus L.)*, isubgol husk *(P. ovata)*, pectin (a water soluble colloidal carbohydrate of high molecular weight found in ripe fruits, such as apples, plums, and grapefruit, and used to jell various foods, drugs and cosmetics) and rice *(Oryza sativa L.)* powder. Guar gum was obtained from Wahdat Traders, Peshawar (Pakistan) while the remaining agents were procured from the local market. Gelling agents at various concentrations (Table 1) were added to the basal medium and heated for 15 to 20 min to get good solution. Plates containing agar only served as control. 20 ml Luke warm media were then dispensed into each Petri dish under aseptic condition to determine their gelling ability. Gelling ability was assessed only by rotating the Petri dish containing the medium after 24 h.

Suitability for fungal growth

Gelling agents yielding promising results were then used in microbial growth studies. *Trichoderma harzianum, Alternaria*

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

Gelling agent	Solidification/gel	Remark
Agar	+	Solidified
Arrowroot	-	Runny
Coconut powder	-	Runny
Corn flour	-	Lumps
Gel rite	-	Runny
Glue	-	Lumps
Gond katira	-	Lumps
Guar gum	+	Solidified
Husk	-	Lumps
Pectin	-	Lumps
Rice powder	-	Runny

+, Solidification; -, lack of solidification.

alternata and *Alternaria solani* served as test fungi to check the efficacy of agar substitutes to support fungal growth. A 5 mm plug of actively growing colonies of each fungus was plated separately in the center of each plate under aseptic condition. Plates were incubated at 25°C for 1 week. Colonies were then examined for various growth parameters such as radial growth, number of spores ml^{-1}, biomass and cost benefit ratio. Radial growth was recorded by measuring the colony diameter along two perpendicular lines and then taking the mean of the two measurements. Number of spores ml^{-1} was counted by flooding the plates with 10 ml of sterile distilled water and scrapping the colony surface with a rubber spatula. The conidial suspension was then filtered through cheese cloth to remove as many mycelial fragments. The suspension thus obtained was used for spore count using a haemocytometer.

For biomass production, three mycelial plugs of the test fungus from 1 week old cultures were placed equidistantly in an Erlenmeyer flask (250 ml) containing 100 ml of the test media. The flasks were sealed with sterile cotton plugs and incubated at 25°C for two weeks. Following incubation, the cultures were harvested from each replicate. The fungal yield was then determined by collecting the fungal biomass on pre-weighed filter papers. The studies were repeated at least twice.

Cost benefit ratio

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

RESULTS AND DISCUSSION

Among agar substitutes tested, guar gum at 2.8% was the only gelling agent that gave promising results when compared to agar. The other agents were either runny or so thick that they formed lumps soon after autoclaving and well before pouring the media into Petri plates (Table 1). Plates fortified with guar gum, on the other hand, were rock solid with clear consistency.

Guar gum is obtained from guar bean or cluster bean (*Cyamopsis tetragonoloba*) 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 arid conditions (Overbeeke et al., 1990). The guar seeds are dehusked, milled and screened to obtain guar gum. It is 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 polysaccharide 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 concentration (Goldstein and Alter, 1973). It is economical since it has almost eight times the water-thickening potency of cornstarch. Further, only a minimal amount is required for obtaining sufficient viscosity (Brown and Livesey, 1994).

Guar gum has been used previously as a substitute of agar, mainly for plant tissue culture media (Jain et al., 2005). The present studies however, focused on its role as a substitute of agar in microbiological culture media. Maximum radial growth of *A. alternata* and *T. harzianum* measuring 7 cm was recorded on the medium fortified with guar gum which was 34.6 and 2.94% more than agar amended media. Colony diameter of *A. solani* was however less on guar gum amended media compared with agar amended media (Table 2).

Maximum number of spores ml^{-1} of *A. solani* (6.7×10^5) and *T. harzianum* (1.2×10^6) were obtained on media fortified with gaur gum which was 9.83 and 96.72% more than agar amended media. Number of spores of *A. alternata* however on the media gelled with guar gum were 1.6% less than that to agar amended media (Table 3). Similarly, maximum biomass of *A. alternata* (1.8 g), *A. solani* (1.6 g) and *T. harzianum* (2.1 g) was recorded on the medium fortified with guar gum which was 200, 176 and 188% more for the stated fungi in that order than agar amended media (Table 3). Its beneficial influence on the growth of fungi could have been due to the presence of a colloidal polysaccharide which is composed of

Table 3. Radial growth, number of spores and biomass of the three different fungi on media gelled with guar gum and agar.

Test fungus	Radial growth (cm)		Spores ml^{-1}		Biomass (g)	
	Agar	Guar gum	Agar	Guar gum	Agar	Guar gum
A. alternata	5.2	7	6.3×10^5	6.2×10^5	0.6	1.8
A. solani	5.8	5.4	6.1×10^5	6.7×10^5	0.58	1.6
T. harzianum	6.8	7	6.1×10^5	1.2×10^6	0.73	2.1

Table 4. Cost benefit ratio of agar and guar gum.

Gelling agent	Concentration (%)	Cost/pack ($) Kg	Cost/liter ($)
Agar	2	58	1.17
Guar gum	2.8	2	0.005

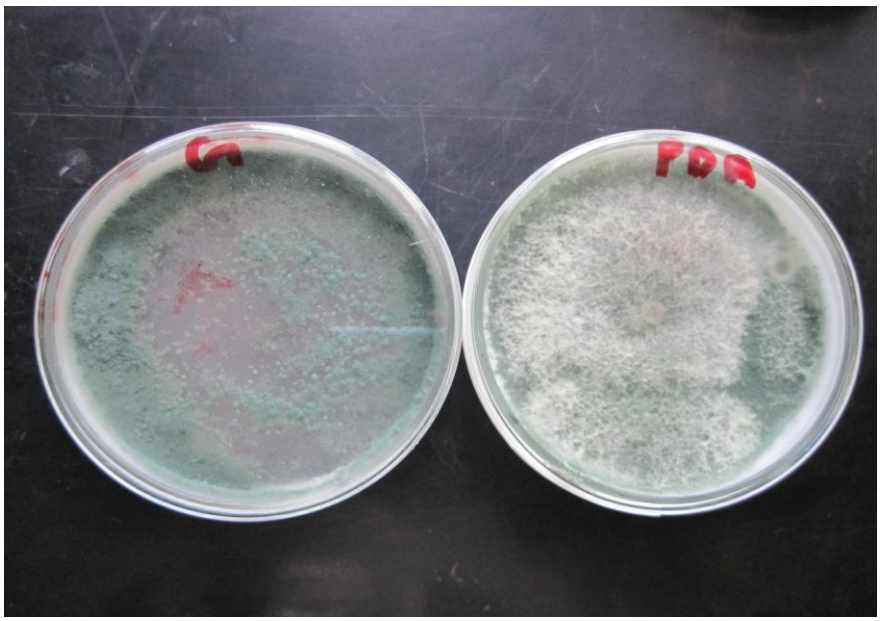

Figure 1. Growth of *T. harzianum* on guar gum and PDA.

straight chain mannan, and a galactose residue attached to every second mannose molecule (Goldstein and Alter, 1973). However, this needs to be substantiated.

From the foregoing, the universal acceptance of guar gum as an alternative gelling agent becomes evident that the problems which may prevent it from its inferior gelling quality, low clarity of agar and metabolizable nature will lead to softening of the media during the culture period. There will be no adverse effect on the growth of microorganisms in the presence of guar gum. The properties of 'guar gum', including its polysaccharidic 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. In comparison, the media gelled with the agar used in the present study appeared almost opaque.

The financial constraints in research encountered commonly in under developed countries like Pakistan warrant that a cheap alternative be sought. Guar gum, the gelling agent used in the present study, unlike agar absorbs water even at room temperature that results in its gel formation. Guar gum being 20 times cheaper than agar could prove to be a cheap alternative (Table 4). Its source, *C. tetragonoloba*, is an easily cultivable plant, and therefore, increase in its demand could be easily met by increasing the area of cultivation.

Representative cultures of fungi on media gelled with agar (right) and guar gum (left) has been shown. Figure 1 shows the comparison between the growth of *T.*

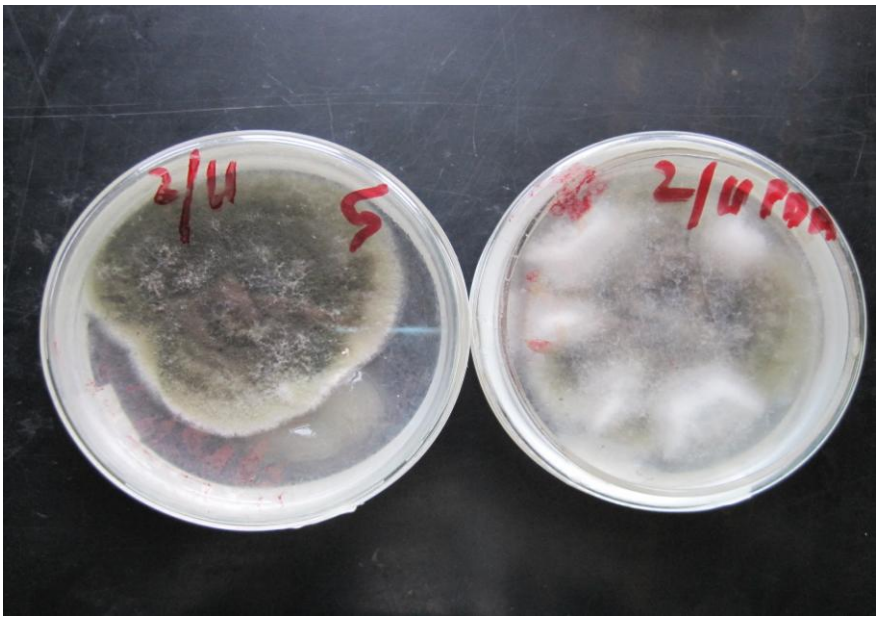

Figure 2. Growth of *Alternaria* spp on guar gum and PDA.

Figure 3. Growth of *Alternaria* spp on guar gum and PDA.

harzianum on potato dextrose agar (PDA) media and guar gum media and Figures 2 and 3 show the comparison between the growth of *Alternaria* spp on PDA media and guar gum media.

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