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VEGETABLE WASTE AS ALTERNATIVE MICROBIOLOGICAL MEDIA FOR LABORATORY AND INDUSTRY

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ABSTRACT

The present study was aimed at finding the potential of vegetable waste as a culture media. Composition of the media and the efficiency of the media to support microbial growth were tested. The medium had a lower protein content and higher carbohydrate content. The media pH was 6.8 before sterilization. Medium supported growth of bacteria, fungi and yeast. Growth was comparable to that obtained on routine commercial media.

KEYWORDS: Vegetable waste, GCO, microbiological media, cost effective.

INTRODUCTION

A growth medium is a liquid or gel designed to support the growth of microorganisms. The commercially available media are very costly. Routine practical require large amount of media on regular basis for streak plate, pour plate, spread plate experiments. Availability of low cost media rich in nutrients, giving comparative results is the NEED OF THE DAY. The search for alternative, cheap media for use in laboratory agents for routine microbiological experiments is going on. Recent research has been focused on finding alternatives to gelling agents of media, agar in particular, and media, in general, because of its exorbitant price.[1-5]

Mondal *et.al*[6] used cucumber and orange peels to evaluate the production of single cell protein using *Saccharomyces cerevisiae* by submerged fermentation. The authors state that

the bioconversion of fruit wastes into single cell protein production has the potential to solve the worldwide food protein deficiency by obtaining an economical product for food and feed. Fruit wastes rich in carbohydrate content and other basic nutrients could support microbial growth. Apple, turnip, papaya and banana peels were used for alcohol fermentation and biomass production by Kondari and Gupta (2012).[7] The use of legume seeds as alternative nutrient media for bacteria and fungi has been reported.[8-10]

MATERIALS AND METHODS

Raw material and processing

- Vegetable waste : a) Onion peels, b) Garlic peels, c) Corn peels
- Dry the peels for 2-3 day in sunlight, separately.
- Grind the peels to powder.
- Use above powders in appropriate proportion to prepare media.

Preparation of Garlic Corn Onion (GCO) Media

1g of the onion powder +0.5g corn powder +0.5g garlic powder +100 ml distilled water. Stand the mixture for 30 min & then filter. Add 0.05g yeast extract + 2g agar. Sterilize. To prepare liquid media, agar was omitted out.

Growth of microorganisms

Bacterial & yeast cultures were streaked on the different media plates by quadrant streak method. *Bacillus* sp., *Sarcina* sp., *Pseudomonas aeroginosa, Candida albicans, Saccharomyces cerevisiae* were used for streaking. Fungal cultures which included, *Penicillium chrysogenum, Aspergillus niger* and *Trichoderma viridae*, were spot inoculated in the center of the plates. Plates were incubated at ambient temperatures. Liquid media were also inoculated with bacterial, yeast and fungal cultures. The tubes were incubated at room temperature for 48 – 72 hours. Colony characteristics of the bacterial cultures were studied by streak plate method on GCO agar and nutrient agar. Growth and sporulation time of fungal cultures on GCO agar were studied by spot inoculating the fungal spore suspensions on GCO agar plate.

Estimation of protein and carbohydrates

Protein was estimated by Folin Lowry's method while carbohydrate content was analysed by DNSA method.

RESULTS AND DISCUSSION

GCO media supported the growth of bacteria, yeast as well as fungi. There was no significant variation in the colony morphology of bacterial and yeast cultures. The pigmentation was slightly affected in case of *Pseudomonas* sp. (Fig.1) but not in *Sarcina* sp. The extracellular pigment might be reacting with the media components leading to the change in colour of the pigment produced by *Pseudomonas* sp. intracellular pigment of *Sarcina* sp. remains unaffected. No variation was observed in the growth of yeast *Saccharomyces cerevisiae* (Fig.2) as well as *Candida* sp. (Fig. 3) on Sabauraud's agar and GCO agar media.

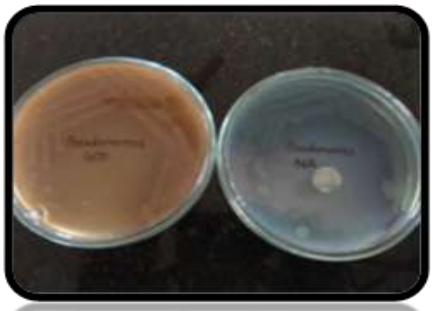

Fig.1a *Pseudomonas aeruginosa* on GCO

Fig.2 *Saccharomyces* spp. On Nutrient and GCO media

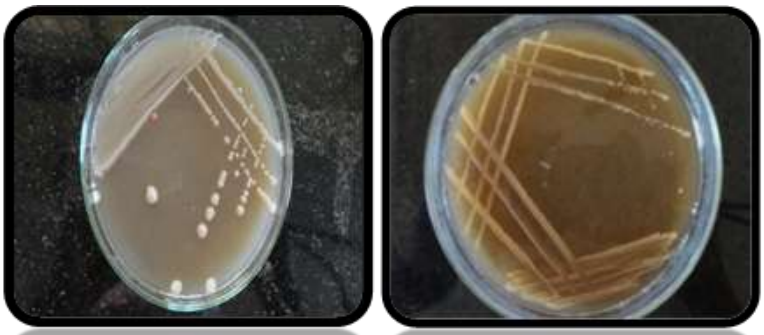

Fig.3 *Candida* spp. On Nutrient and GCO Media

The fungal culture grew better in GCO media. On solid media, the sporulation was faster as compared to regularly used media. In liquid media also, faster mycelial growth was observed. Good growth was obtained after 24 h of incubation as seen in Fig. 4. On further incubation, sporulation was seen of culture inoculated on GCO media. Thus, on GCO media, sporulation took place within 48 hrs while on Sabauraud's agar sporulation was seen after 72 hrs. This shows that GCO media is very suitable for growth of fungal cultures.

Fig.4 *Trichoderma* spp. on GCO media

Fig.5 *Penicillium* spp. on GCO media

Fig.6 *Aspergillus* spp. on Sabauraud's and GCO media

The protein and sugar composition was estimated by standard methods and showed presence of 24.2 mg/dl and 28 mg/dl of sugar and protein, respectively. Thus, being rich in these components, the media is able to support growth of microorganisms.

Table I: Concentration of sugar and protein in the GCO media

Test	Nutrient	Content
DNSA	Reducing Sugar (Glucose)	24.2 mg/dl
Folin-Lowry Method	Total Protein	28 mg/dl

Carbohydrate rich media has been used by other workers as alternative media for the growth of fungi. Sago and palmyrah tumor media was used for fungal growth.[10] Protein and carbohydrate rich raw materials like Soya, Potato, dates, Groundnut, Cereals, Cassava, Yam, Pigeon pea, Maize and Beans have been successfully used in formulation of cheap alternative bacteriological media.[11-16] A media formulated using food wastes that included defatted soya, clear beer spent yeast, potato solid waste and opaque beer spent grains, containing ampicillin, isopropyl ß-D-1-thiogalactopyranoside and 5-bromo-4- chloro-3-indolyl-ß-D-galactoside proved to be an equally alternative medium in molecular biology for selection and screening *E. coli* TG1 cells transformed with pUC18 plasmid.[17]

The growth results obtained using GCO media prove its efficiency as a alternative cheap media for routine microbiological experimentation. Similar finding have been reported. *Curvularia lunata, Aspergillus niger, Aspergillus flavus, and Fusarium oxyaporum* were screened initially for growth in 16 different plants extracts broth media.[18] GCO media supports fungal growth with short sporulation time. This is favourable for production of *Trichoderma* sp. on large scale. This media thus can be a promising media for growth of industrially important fungi. Industry requires that the producer organism grows faster, so that production time is reduced which is economical. Similarly, low cost media is always sought after again to bring down production cost.

As shown in Table II, the cost of GCO liquid media is drastically less than the commercial liquid media. The GCO media containing agar for solidification is also very cost effective as compared to commercial solid media. The rise in the cost of GCO agar media is due to incorporation of agar, which adds on to the cost. Alternative cheap source of solidifying agent needs to be found so that this cost may be brought down.

Table II. Economical comparison of the media

Media type	Cost in Rs/ 100L	
	Commercial media	'GCO' Media
Solid media	12800	9700
Liquid Media	3180	80

This media was formulated as an alternative to the costly commercial media that is used for routine experiments in the laboratory. Thus it could replace the media for practicals such as enumeration of bacteria by pour plate, spread plate methods, streaking methods, etc. This has cut down the expenditure on media, significantly. Similarly, the efficiency of the media as

production media for fungal based fermentations needs to be checked. Attempts have been made at replacing the industrial media by cheap alternative vegetable based or agri-based media for production of biomass, biopolymer, citric acid.[6,18-19]

CONCLUSIONS

Vegetable waste was used to formulate a cost effective media. Media with minimal ingredients was formulated & named GCO media. The growth of different microorganisms was comparable with regular media. Economical comparison shows the GCO media to be highly cost effective. GCO is good alternative media for laboratory use. Can be used at industrial level for fungal based productions.

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