Kitchen Waste Agar: A Novel Media For Fungal Cultivation

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ABSTRACT:

Use of dehydrated media is of utmost importance in the field of microbiology for cultivation of industrially important organisms. Instead of using high cost microbial culture media, kitchen waste material could prove to be a good alternate source for the production of low cost culture media. The wet waste generated in kitchen includes vegetables & fruit waste. It is a waste that almost every house generates every day. It can serve as a good source of nutrients & vitamins for microorganisms. Hence these materials can be used to formulate solid media for the growth of bacteria and fungi. In the current study, kitchen waste like pomegranate peels, orange & sweet lime pomace, spinach stems & pea pods have been included to formulate the new media. Comparing the growth of organisms on conventional and newly formulated kitchen waste agar, it was found that the media prepared from kitchen waste serves as a good and inexpensive source of nutrients for many bacteria and fungi. Thus, it can be further used commercially for isolation and cultivation of various microorganisms.

Keywords: Kitchen waste, Agar, Media, Cultivation of organisms, Fungal

INTRODUCTION:

Culture media is of prime importance in the field of microbiology and applied sciences. Any growth medium in its liquid or solid form is used to grow, enrich, observe and quantify microorganisms. On solid media one can observe the peculiar colony characteristics shown by an organism while in liquid the organism can be enriched to obtain high yield of the microbial product. Various commercial media are available for this purpose to suit the growth of the particular type of microorganism. However such media are costly. Scientists are striving continuously for searching an alternative way to design a cost effective medium that can facilitate the growth of the microbes.

Waste management is a problem all over the world. Especially in developing countries like India, increasing waste and managing its disposal is posing a serious threat. The waste generation in urban areas of India is approximately 170000 tonnes per day. About 40 – 60% of waste generated in urban through household practices. [15] The waste can be divided into two forms: Dry waste and Wet waste. Wet waste mostly contains organic waste. Approximately 2-3 thousand tonnes of organic kitchen waste are generated per year. Wastes can be an effective resource for recovery of recyclable materials.

Microorganisms require nutrients as source of energy for their metabolic processes in order to grow and reproduce. Wet organic waste generated from kitchen includes fruits and vegetable wastes which is rich in essential nutrients required for microbial growth. Thus other than formulating compost from organic waste, it can serve as an alternative source for the cultivation of various microorganisms. This can be a cheaper source for the formulation of growth media. It is observed that wet organic kitchen waste serves as a better source of nutrients essential for fungal growth. Waste generated from fruits and vegetables includes peels, seeds, pomace, stems, pods, etc. Such wastes are rich in carbohydrates and other nutrients which are easily utilized by microorganisms. [12]

The problem of costly commercial media can be overcome by using natural media prepared by using kitchen waste. Standard Procedures in any microbiological labs require large amount of media for techniques like isolation, enrichment, spread plate technique and several other experiments. Thus low cost media rich in nutrients, giving reproducible result is need of the day.

METHOD AND MATERIALS:

Procurement of raw materials: Kitchen waste materials like pomegranate peels, sweet lime and orange pomace (leftover after extraction of juices), peapods and spinach stems were procured from household waste and local market. One kg of each waste material was collected. Samples were brought to the laboratory using sterile plastic bags for further processing.

Processing of waste material: Seeds were separated from pomace. Pomegranate peels, peapods and spinach stems were washed two to three times with sterile distilled water to remove any dust or soil particles. They were then cut into small pieces using a sterile knife.

Preparation of powder: The raw materials were dried for 48 hrs at 80°C in a hot air oven. The dried raw materials were ground by using kitchen grinder to obtain its powdered form. Each powder was sieved to obtain finer particles of the powder and then stored in clean and dry plastic containers.

Preparation of media: One gm of each powder was added in 20 ml of warm distilled water and kept overnight for obtaining its natural extract. Each solution was then filtered and 5 ml of this filtrate was taken in conical flask. The volume was made up to 100 ml using distilled water. pH of the media was adjusted by using pH meter. 3% of agar powder was added to the solution for solidification. Media was autoclaved and poured into sterile petriplates.

Isolation of fungal species: Four different fungal species viz. *Aspergillus spp., Penicillium spp., Candida albicans* and *Saccharomyces cerevisiae* were taken as standard fungal cultures from an undergraduate laboratory of Microbiology & Biotechnology, VPM's B. N. Bandodkar College of Science. These organisms were isolated on newly prepared kitchen waste agar as well as commercially available Sabouraud's Agar (Prepared by mixing the components obtained from HiMedia, Mumbai) using side streak and T-streak method. The plates were incubated at room temperature for 24-48 hrs. The obtained growth was compared.

Estimation of carbohydrate and protein content: The concentration of reducing sugar and protein in newly formulated Kitchen Waste Media is estimated by using quantitative methods like DNSA (Dinitrosalicylic Acid) and Folin-Lowry. For DNSA, 1 ml of media broth and 1 ml of DNSA reagent was mixed and subjected to boiling water broth for 10 minutes. After cooling, 8 ml of distilled water was added. The quantity of reducing sugar present in the broth was calculated using standard graph plotted by taking glucose of 1000 µg/ml as standard (Range 200-1000 µg/ml). Absorbance was measured colorimetrically at 530nm. The Folin-Lowry method involved colorimetric estimation of proteins in the broth using BSA (200µg/ml) as standard protein (Range 40-200 µg/ml). 1 ml of broth was mixed with 5 ml of alkaline $CuSO_4$. This was followed by 10 minutes incubation and addition of 1:2 diluted FC reagent. The resulting colour change after 30 minutes of incubation was used for colorimetric estimation.

Analysis of cost-effectiveness of KW agar: The cost effectiveness of KW media was analyzed in comparison to the commercially available media with reference to the results obtained.

RESULTS:

Procuremet, Processing and Preparation of Powdered form of raw waste materials:

Fig.1.a. Spinach stems and its dry powder

Fig.1.b. Peapods and its dry powder

As shown in the figure above, all the raw materials were procured and processed to obtain powdered form. The newly formulated Kitchen Waste (KW) media supported the growth of fungi. In fact the fungal growth was faster on Kitchen Waste agar as compared to commercially available Sabouraud's media. All the standard fungal cultures showed growth after 24 hrs on this media while on Sabouraud's agar took 48 hrs to show the growth, at room temperature. As shown in the figures below, *Aspergillus spp.* (Fig.2), *Penicillium spp.* (Fig.3), *Candida albicans* (Fig.4) and *Saccharomyces cerevisiae* (Fig.5) showed luxuriant growth within 24 hrs.

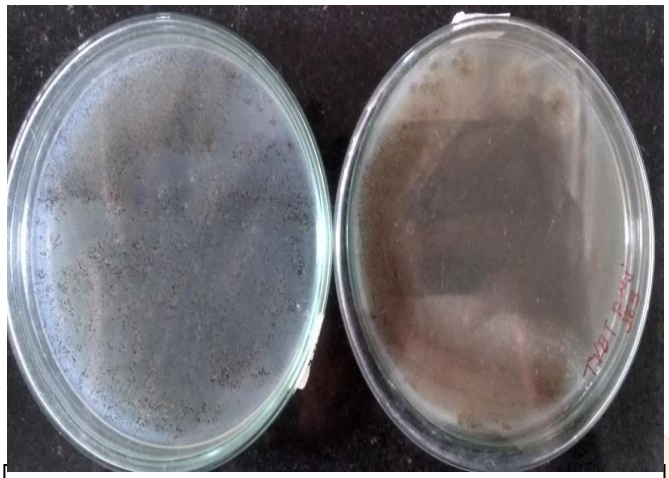

Fig.2 *Aspergillus spp.* on Sabouraud's and KW agar

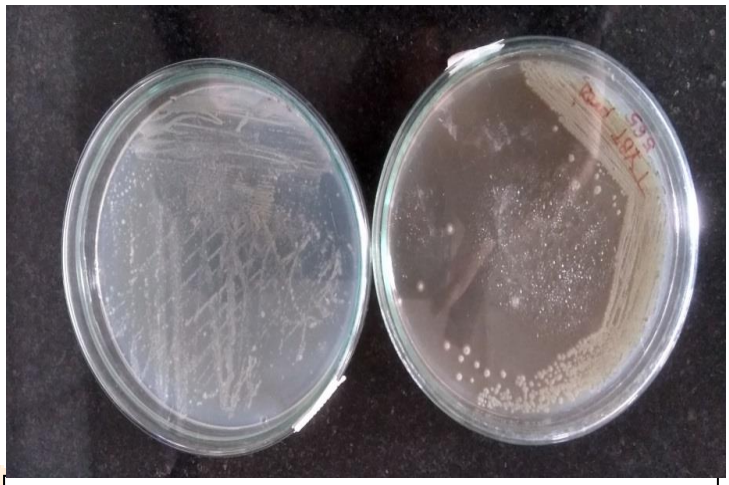

Fig.3 *Penicillium spp.* on Sabouraud's and KW agar

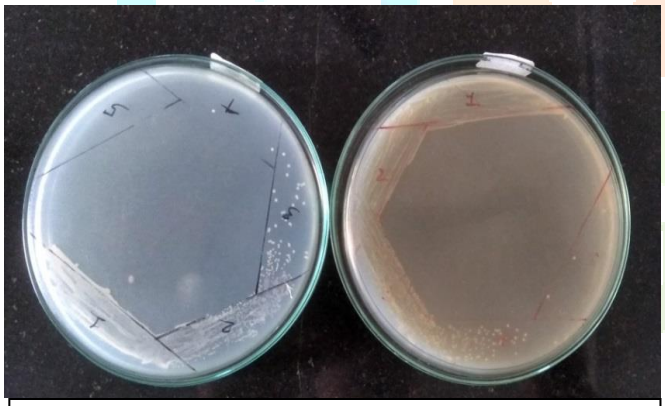

Fig.4 *Candida albicans* on Sabouraud's and KW agar

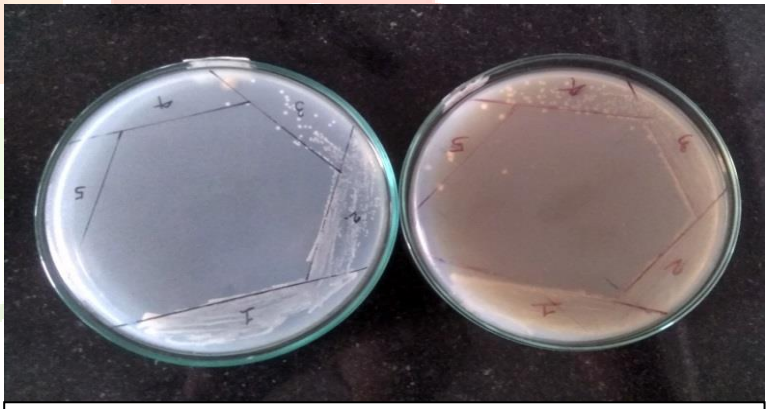

Fig 5. *Saccharomyces cerevisiae* on Sabouraud's and KW agar

From the results obtained by performing folin-lowry method and DNSA method, it was observed that the media contained higher protein content than carbohydrate as shown in the table below.

Table 1: Carbohydrate and protein content of newly designed KW broth

TEST	NUTRIENT	CONCENTRATION
Folin Lowry method	Protein	1.5 mg/ml
DNSA method	Reducing Sugar	0.8mg/ml

Economical analysis of the media:

Many industries require large amount of dehydrated media for cultivation of industrially important organism within short time on large scale & also the commercially available media used for microbial cultivation is very much costly that it may not be affordable for lab purpose. There are several studies which have tried replacing commercial media with cheap alternative media. Considering the commercial media, 100 gms of

Sabouraud's Dextrose Agar powder costs approx. Rs. 1000/- in India. Thus 1 kg of this media costs around Rs. 9 to 10K. The agar agar powder of 500 gms quantity cost for approximately Rs. 4000/- in India. Considering the cost of agar agar powder used to formulate the kitchen waste agar and all the procedures involved in it, it can be easily estimated that the kitchen waste agar is a way cheaper than commercial media.

DISCUSSION:

Cultivation of industrially important microorganisms requires large amount of growth media. Researchers across the world are working on replacing high-cost media with cheap alternative media. Scientists have also concluded that media formulated using organic waste can prove to be the best replacement for the high-cost commercial media. GCO media was formulated by Berde et al using vegetable waste such as onion peels, garlic peels and corn peels. It supported the growth of bacteria, yeast as well as fungi in very less time as compared to commercially available growth media. Anbu et al showed that fruit peels of pineapple, Mango, jackfruit, Green banana, Yellow banana, sweet lime and Pomegranate have been used in formulation of cheap alternative media which supports the growth of bacteria as well as fungi. Various research articles have proved that Lignocellulose waste serves as a good source of cellulose production. Apple pomace, potato peels, rice, leguminous seeds, defatted soya etc. are rich in carbohydrate and protein content and serve as good source for formulation of alternate media. (Attri et al, Antony et al)

CONCLUSION:

Waste recycling is the best method of waste management. In the current study, Kitchen waste was used to formulate a dehydrated medium that can be used in every day undergraduate laboratory practices for fungal growth. The newly synthesized medium was named Kitchen Waste Media. When compared for the growth of the fungal cultures with that of commercially available media, it was observed that the test organisms grew luxuriantly on this medium as they did on commercially available media in the market. In fact, it took only 24 hours of incubation for these organisms to grow on Kitchen Waste Agar, unlike the Sabouraud's media that requires 48 hours. Thus use of this medium can prove to be very cost effective and environmental friendly.

FUTURE PROSPECTS:

- Different sources of the kitchen waste other than used in current research, their proportionate composition, pH and temperature regimes can be standardized to make it suitable for growing bacterial cultures to replace media available for bacterial growth like nutrient agar.
- The media formulation can be taken up as an entrepreneurial practice and can contribute to the industrial development of the country and in employment generation.

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