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Research Article

Formulation and Evaluation of A Microbial Growth Media from Kitchen Waste: A Cost-Effective Alternative for Commercial Media

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Abstract

The study was carried out to formulate and evaluate microbial growth media using wasted rice water and onion peel. In our study, *Aspergillus niger* was cultivated on our media at different pH (4, 7 & 10) and observed better growth at acidic pH (4). *Aspergillus brasilensis, Mucor, Coccidioides, Fusarium oxysporum* were cultured at acidic pH on our medium. After 48 hours of incubation, good growth was observed in rice water onion peel media. The nutrient quality of the media was determined using Lowry method and DNS method. The protein content and reducing sugar content were 0.03 mg/ml. The protein content was less for our media compared to nutrient broth. Thus, from our study, we concluded that the prepared media can be used to cultivate fungi in labs. The nutrient quality of the media can be increased by adding different nutrients (Yeast extract, Peptone. Dextrose etc.), which may aid in supporting the growth of bacteria and yeasts in the medium.

Keywords: *Food waste, Agar, Alternative media, Fungal Growth medium, Culturing fungi.*

Introduction

Food waste is a term that refers to food products which are of interest for consumption but have been intentionally discarded. Wasted food has an extensive effect, both nationally and globally, upto 40% of all food produced goes uneaten and about 95% of discarded food ends up in landfills (Parfitt, *et al.*, 2010). The proper use of food waste and by-products as raw materials or additives could lead to economic benefits for the industry, reduce nutritional problems, improve health, and reduce environmental repercussions resulting from improper waste management.

Microbial culture media can be prepared from food waste rich in essential nutrients that are required for microbial growth. Routine culturing of microorganisms requires a higher amount of media rich in nutrients. Recent research has been focused on finding alternatives to gelling agents of media, agar in particular and media in general because of excessive price. Food wastes like potato peels, lettuce, cooked meat, pumpkin peels, tomatoes, egg shells and mushroom broth are used for culturing fungi (Chalon, *et al.*, 2013). Rice (*Oryza sativa*) water and onion (*Allium cepa*) peel are the most common kitchen waste of the households of Kerala, India. Rice grains constitute 70% - 80% starch and 7%-10% protein and also contain many types of vitamins and minerals like vitamin B and vitamin E, anti-oxidants and amino acids (Murugeswari, *et al.*, 2020). Onion peel contains 2.5% protein, 19.17% carbohydrate, 0.77% fat of its dry mass (Michalak-Majewska, *et al.*, 2020). Preparing a growth media using rice water and onion peel is an innovative way to reduce kitchen waste and provide a low-cost media for laboratory purposes such as routine culturing, fermentation processes etc.

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Materials and Methods

Preparation of Media

Cooked rice water and onion peel for 5 days were collected and stored in the refrigerator until used. Later the collected rice water was boiled and made into a thick paste, spread on butter paper and dried in hot air oven and powdered. The collected onion peel was dried using hot air oven and powdered. Later the rice water powder and onion peel powder were mixed together to prepare the rice Water - Onion peel media.

Optimization of pH of the Media

The Rice water-Onion peel media and 1.5 percent (%) agar-agar was weighed and suspended in 20 millilitres (ml) of distilled water (DW). The pH of Rice water-Onion peel media in three different boiling tubes was adjusted to 4, 7, and 10 using 1 normal (N) NaOH and 1N HCl (Child, *et al*., 1973) and autoclaved at 121 degree Celsius (^{o}C) & 15 load balance system (lbs) pressure for 15 minutes (mins). The prepared media was poured into the petri dishes and allowed to solidify. The test organism *Aspergillus niger* (*A. niger*) was inoculated onto three plates using an inoculation loop by streaking. The plates were then incubated at room temperature (RT) for 48 hours (hrs).

Test Culturing of Known Fungal Species

The test organisms *Aspergillus brasiliensis (A. brasiliensis), Fusarium oxysporium (F. oxysporium), Mucor,* and *Coccidioides* were inoculated onto the media by streaking. Later the plate containing *A. brasiliensis* was incubated at 37^{o}C in an incubator and the other three plates were incubated at RT for 48 hrs.

Alkaline Hydrolysis of the Media

Alkaline hydrolysis was carried out following the procedure of Zvidzai, *et al*., (2007) with modifications, to make the media completely dissolved in water to carry out the nutrient content estimation studies. The prepared media was mixed with 3 Molar (M) NaOH solution and kept at 80^{o}C in the water bath for 24 hrs. Later the pH was adjusted to 7 using concentrated HCl. This mixture was then centrifuged at 4000 rotations per minute (rpm) for 15 mins. Later the filtrate was collected and dried at 50^{o}C in hot air oven. Finally powdered and kept inside the refrigerator at 4^{o}C.

Protein Estimation by Lowry Method

Different concentrations of the standard Bovine Serum Albumin (BSA) were prepared (1mg/ml- 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1ml) and made to 1 ml using DDW. 5ml of pre-prepared alkaline copper sulphate solution was added to each test tubes and allowed to stand for 10 mins. After 10 mins 0.5ml of Folin-Ciocalteu (FC) reagent was added to each tube and incubated at RT for 30 mins in a dark room. Later the absorbance was measured at 670 nanometers (nm). The hydrolyzed sample of media and Nutrient Broth (NB) were prepared, the same was repeated for standard. A graph was plotted with the absorbance values obtained.

Reducing Sugar Estimation by DNS Method

Different concentrations of the standard glucose were prepared (1mg/ml- 0.2ml, 0.4ml, 0.6ml, 0.8 ml, 1ml) and made to 1 ml using DW. 1 ml of 3, 5 dinitro salicylic acid (DNS) solution was added to each tube and kept in water bath for 10 mins at 100^{o}C. After cooling down to RT, absorbance was measured at 540 nm. A graph was plotted with the absorbance values obtained. The same steps were repeated for the hydrolyzed sample of the media (Daptardar, *et al*., 2018).

Results

Media Preparation

The Rice water – Onion peel media was prepared in dehydrated form and stored at room temperature.

Fig. 1: Dehydrated Rice Water - Onion peel media

Optimization of pH of the Media.

After 48hrs of incubation at 37°C in an incubator, better fungal growth was observed at pH 4 and pH 7. Out of 2 pH, slightly better vegetative growth was observed at acidic pH 4. The results are given in Fig 2.

Fig. 2: The growth of *Aspergillus niger* on rice water-onion peel media on different pH (A- pH 4, B-pH 7, C- pH 10)

Test Culturing of Known Fungal Species

The test organisms *A.brasiliensis, F .oxysporium, Mucor, and Coccidioides* showed good growth after incubation for 48 hrs of incubation. The results are depicted in Fig 3.

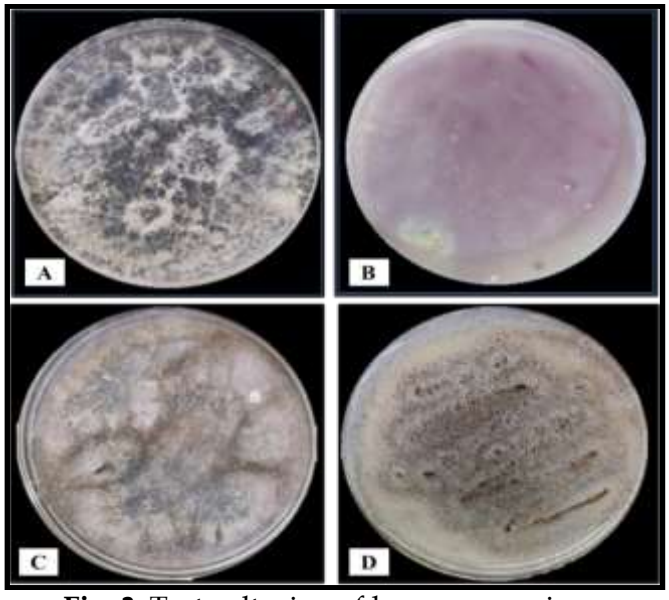

Fig. 3: Test culturing of known organisms
A - *Mucor*, B - *F. oxysporum*, C - *Coccidioides*, D - *A. brasilensis*

Alkaline Hydrolysis

After alkaline hydrolysis Rice water - Onion peel medium became water soluble and transformed into off white in colour.

Fig. 4: Rice-Water onion peels media Hydrolysate.

Estimation of Protein and Reducing sugar

Estimation of protein in the media was carried out by Lowry method using BSA and NB as reference standards. The rate of absorbance of all three samples depending on the concentration is given below in table 1 and Figure 5.

The protein content of Rice water - Onion peel media hydrolysate was found to be slightly lower than NB (NB ≈ 0.06 mg/ml & Rice water-Onion peel media ≈0.03 mg/ml).

Estimation of reducing sugar content in the media was carried out by DNS method using glucose as the reference standard. The rate of absorbance of glucose and Rice water - Onion peel media depending on concentration is given below in Table 2 and Figure 6.

The reducing sugar content of Rice water - Onion peel media was found to be ≈ 0.04mg/ml.

Table 1:- The absorbance of BSA, NB and Rice water-Onion peel media at different concentrations (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1 mg/ml)

CONCENTRATION (mg/ml)	ABSORBANCE		
	BSA	NB	RICE WATER-ONION PEEL MEDIA
0.2	1.266	0.092	0.071
0.4	1.852	0.128	0.077
0.6	2.409	0.142	0.086
0.8	2.719	0.163	0.0.87
1.0	2.770	0.165	0.090

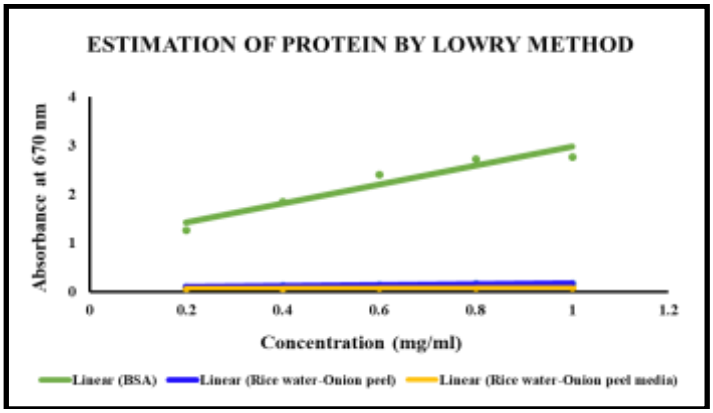

Fig. 5: Protein estimation of BSA, NB and Rice water -onion peel media at different concentrations (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml,1 mg/ml)

Table 2:- The absorbance of glucose and Rice water-Onion peel at different concentrations (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1 mg/ml)

CONCENTRATION (mg/ml)	ABSORBANCE	
	GLUCOSE	RICEWATER-ONION PEEL MEDIA
0.2	0.007	0.001
0.4	0.009	0.002
0.6	0.015	0.003
0.8	0.022	0.004
1.0	0.028	0.008

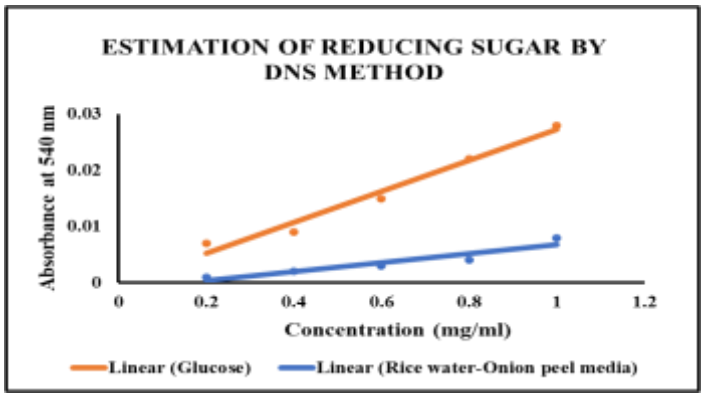

Fig. 6: Reducing sugar content estimation of glucose and Rice water-onion peel media by DNS method at different concentrations (0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8 mg/ml, 1mg/ml)

Discussion

A. niger showed growth at pH 4 & 7, but better vegetative growth observed at pH 4. Simonovicova *et al.*, (2013) also observed better growth of *A. niger* at acidic pH, while studying optimum conditions for enzymatic digestion of polysaccharides. This result is in agreement with our results since our media also contain polysaccharides (from rice water) in the form of starch. Similarly, Daptardar, *et al.*, (2018) showed good fungal (*Fusarium sp., Mucor sp., Penicillium sp., Trichoderma sp.*) growth in media rich in carbohydrates. The test organisms *Mucor*, *F. oxysporum*, *Coccidioides*, *A. brasilensis* exhibited better growth at acidic pH. Daptardar, *et al.*, (2018) also showed that *Aspergillus sp, Pencillium, Canidida albicans* and *Saccharomyces cervisiae* are grown in the acidic pH on the kitchen waste agar. The reducing sugar content of our media was found to be slightly higher than the protein content. Hence our media didn't support the growth of bacteria very well, this may be attributed due to lower protein content in the media. Also, the protein content and reducing sugar content was lower in our media compared to Nutrient agar, a common medium known to grow fungi and bacteria. The reducing sugar content of our media was lower compared to the standard fungal growth media PDA, but it was higher than the protein content present. In a study by Berde & Berde, (2015) adding yeast extract to media made from onion peel supported the growth of bacteria, yeasts and fungi. Similarly adding extra nutrient like peptone, yeast extract would help to upgrade Rice water-onion peel media to grow bacteria and yeast.

Conclusion

In the current study cooked rice water and onion peel, common food waste generated from Kerala households is used to formulate a cheaper alternative for fungal growth media. Different fungal species are successfully cultured in the media. On Nutrient analysis, the reducing sugar content and protein content of our media was found to be less than commercially available media PDA. Even though our media is less in reducing

sugar content than PDA, we managed to observe good growth of different fungal species that are described above. Hence our media could be a good alternative to use commercially for growing fungi, but further optimization and standardization are required to grow other microorganisms such as yeast & bacteria.

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