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Formulation and quality testing of kitchen and temple waste for the production of microbial growth media

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Abstract
Organic material, byproducts produced as a part of remnants in the kitchen and temple, form a huge bulk of useful resources which can be the raw material for the production of several biodegradable nutrition sources.These materials can be used as Biofuel, Fertilizer, manual, compost etc after respective modifications as needed. This method of bio modification and production of useful substances from the waste would not only save the economy but also plays a vital role in cleaning the environment from these waste depositions. The designed work aims to collect the organic remnants from Temple and kitchen and use it for the production of nutrient media required in the culturing of microbes. The actual principle involved in the work is the synchrony in nutritional quality of these wastes and the nutritional requirement for the microbial culture. Further it is necessary to retain the composition with resect to the type of material used in media making irrespective of their exact quantities. Being a Biologically undefined medium it cannot be expected to contain the components in accurate quantities but it becomes vital concept to maintain the quality and type of contents included in the media. Some of the components used include vegetable and fruit wastes, dry flower and leaf material etc. The kitchen waste medium, produced can be used for the growth of fungi and Bacteria while the temple waste media can be used for the cultivation of common bacteria which will be highly economical as compared to costly standard and commercial microbiological media.

Keywords: *Organic, Remnants, Biofuel, Biodegradable, Undefined Medium, Microbiological*

Introduction
With the increasing population of the continent and the ever increasing need of the individuals, generation of lots of vegetable and organic wastes has been dumped into the environment. Additionally the waste organic materials from the temples also add on to the dump and cause environmental pollution [2]. Such a solid waste has to be disposed in a most environment friendly manner without harming the ecological and biological quality of environment. Solid waste disposal has been one of the challenges faced by the decade. In most of the cases this waste is dumped into water bodies causing a threat of water pollution followed by ground water contamination. There are several options proposed to utilize such waste which include: manufacture of compost, fertilizer, biogas production, use of floral waste for scent manufacture etc.[8] However practically this is not yet completely successful.
Nutrient media used in the laboratory for the culture of Bacteria, Fungi and Yeast mostly contains a protein source, micro nutrient source, carbon base and the solidifying agent [3]. Further based on the type of components used media may be of several types. Chemically undefined media contain natural components whose exact chemical content is not known quantitatively in contrast to chemically defined media that contains all calculated quantities of chemicals [1]. In the proposed work use of kitchen and temple waste as a source for the production of microbial media, a chemically undefined media is expected to be formulated except for the addition of solidifying agent, agar. All the basic nutrients required by the organisms are provided in the form of pre treated organic remnants and a media is prepared. The growth of different cultures can be tested and compared to the other regular media.

Materials and Methods

Collection of organic waste from Kitchen and temple:
Every kitchen produces some waste which can be recycled. All humansshould feel it a legal duty to manage the waste. The technique of using kitchen vegetable waste media for the growth of

microorganisms is very simple to implement and is both environment and economy friendly [10]. Kitchen waste which was collected for the preparation of microbial media included the following:
Peels of vegetables like

- Bottle gourd - Lagenaria siceraria
- Potato - Solanum tuberosum
- Tomato waste – Solanum lycopersicum
- Bitter gourd – Momordica charantia
- Ladies finger – Abalmoschus esculentus
- Cucumber – Cucumis sativus
- Brinjal – Solanum melongena
- Fruit waste can also be utilized if available
- Apple cuttings, Banana waste etc.

Fig 1: All the kitchen organic waste collected for the media perpetration:

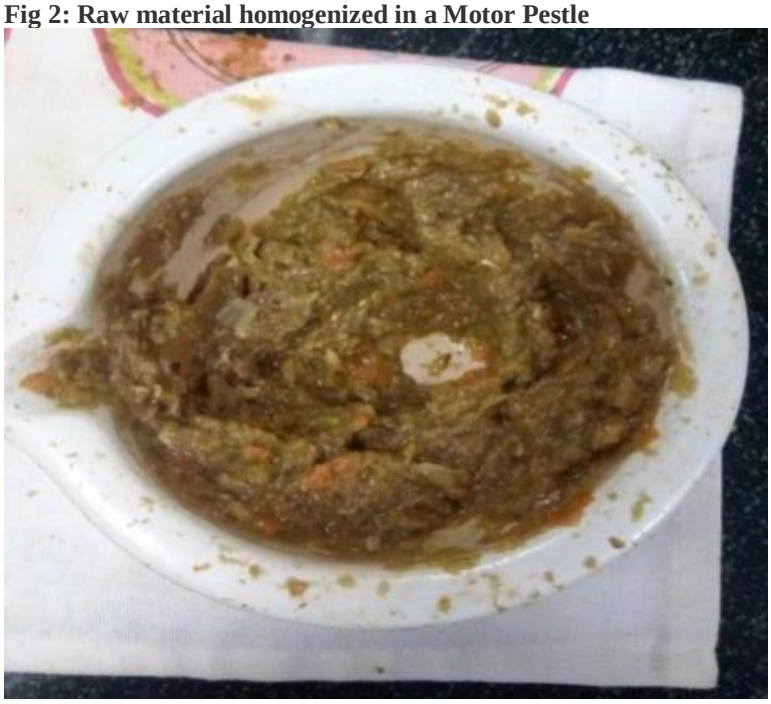
Inference: The above figure 1 shows the beaker containing all the organic kitchen remnants collected for the study, which includes Fruit and vegetable peels and their material.

Treatment of the waste for media formulation

Homogenization of the sample

- After collection of the kitchen waste, 50gms of kitchen waste sample was transferred into a beaker and washed properly fallowed by homogenization of the material. Homogenization is the initial step for media preparation.
- It is performed by using motor and pestle. Homogenization is the process of making the material uniform and smooth paste

Fig 2: Raw material homogenized in a Motor Pestle

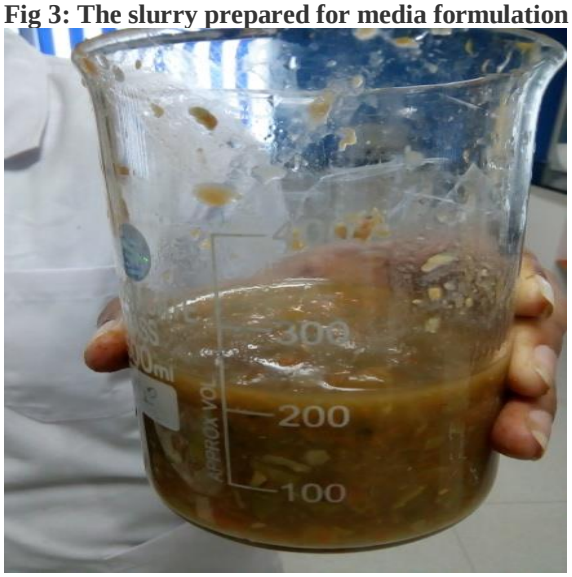


Inference: The above picture shows the homogenized mixture of kitchen waste collected.

Formulation of media:

Once the homogenate is ready it was subjected for further processing. Water was added to the mixture to prepare a slurry followed by boiling as a part of sterilization. After boiling for a period of 5 minutes the slurry was cooled and filtered to remove particulate material and only liquid content was collected. Either muslin cloth or filter paper can be used for this purpose.

Fig 3: The slurry prepared for media formulation



Inference: Beaker showing the slurry of organic vegetable waste which can be filtered further

Fig 4: Ready to use organic extract for the microbial culture

Inference: The liquid filtrate obtained after the boiling and filtering of organic waste. This can further be used for plate making.

Preparation of media from the kitchen waste extract [5]

- Kitchen waste media or the slurry made above was mixed with the prescribed quantity of agar agar for solidification.
- The boiled media was poured into 25ml sterilized Petri plates andthe Petri plates were labelledas A and B.
- Solidification takes place after few minutes.

After solidification the sample was inoculated onto the solidified media under laminar airflow conditions. The plates were incubated as per the regular protocol of 37^0C overnight.
Inoculum used for the study was direct tap water and the volume used was 0.4ml.

Formulation of 2nd media using Temple waste Extract:

Collection of temple waste

Marigold flowers, roses and other types of flowers, leaves etc were collected from various temple waste bins or temple waste sample contains

- Flowers – Marigold (Tagetes)
    - Rose (Rosa)
    - Chrysanthemum
- Mango leaves
- Dhoop stick powder
- Match sticks etc.

Media was prepared from the temple waste using the procedure similar to the above with variability in components used.

Fig 5, 6 : Temple waste homogenate and the ready to use media

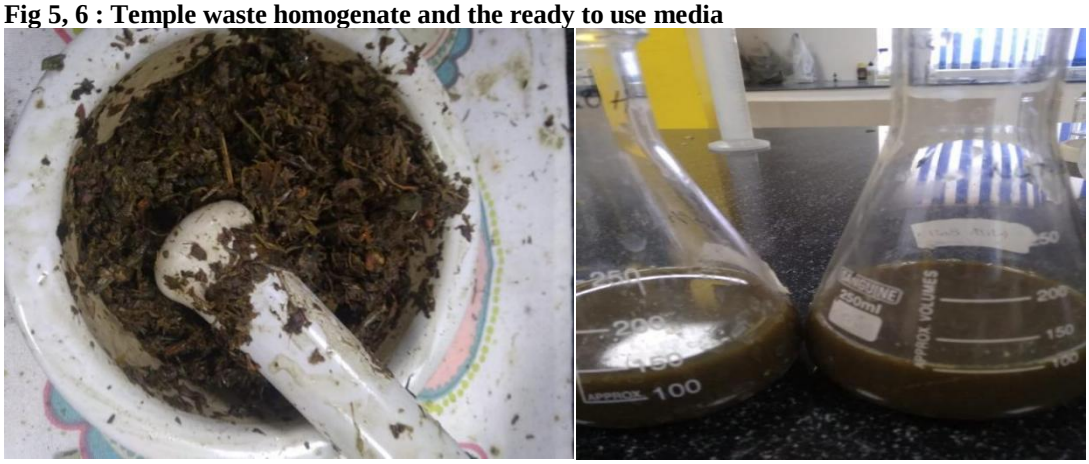


Figure 5 shows the homogenized mixture of temple waste collected. The image 6 shows the extract with water after filtration which can be used for media plate preparation after addition of agar agar.

2.5gms of agar was added for 50 ml of media to ensure solidification followed by microbial culture inoculation.

Perpetration of cultures by inoculation of sample [6]:
After sterilization and solidification 0.4 ml of tap water (inoculum) was used to evenly spread by using a sterile spreader in both temple waste and kitchen waste media plates. The spreading is performed in the laminar air flow followed by incubation in inverted position at 37 degrees for 24 hours.

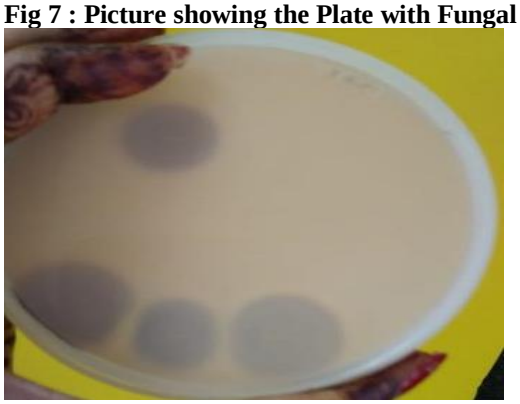
Preparation of pure cultures [7]:
The same media composition was again prepared and used for pure culture preparation of microbial colonies obtained in the previous step. Both the type of media plates were prepared and streaked for obtaining various selected colonies pure culture. All the plates were than incubated under standard conditions overnight.

Identification of Cultures by Gram's staining [9] and Biochemical tests [4]:
All the pure cultures obtained were initially subjected for Grams' staining using standard protocol and based on their gram's nature they were subjected for respective biochemical tests. All the biochemical tests used are listed below along with the identifications.

Results and Discussion

Based on the above study expected results should be the successful growth of diverse bacterial cultures. Thus all the colonies exhibiting morphological variations are subjected for pure culture preparation in the same composition media plates followed by their testing towards identification. The results of Biochemical tests are furnished in the table 1.

Fig 7 : Picture showing the Plate with Fungal growth



The above figure shows the Kitchen waste media with Fugal growth indicating that the media can even be used for fungal isolation with proper use of antibiotics in the formulation to prevent bacterial colonies.

Fig 8, 9 : Before and after Bacterial growth on the Master plates made from Kitchen waste media

Above plate is the kitchen waste media formulation showing before the growth and after the dense growth of bacterial colonies indicating its efficacy and use.

Table 1: All the biochemical tests and results for the Identification of pure cultures

Pure culture	Gram staining	Spore type	Shape	Starch hydrolysis	Catalase test	Citrate test	Gelatin test	Glucose fermentation test	Mannitol test	VP test	NaCl test
P 1	+ve	+ve	Rod	Starch -ve	Catalase – ve	Cit -ve	-ve	NA	NA	NA	NA
P2	+ve	+ve	Rod	Starch +ve	NA	NA	NA	NA	NA	-ve	-ve
P3	+ve	+ve	Rod	Starch -ve	Catalase – ve	Cit -ve	-ve	NA	NA	NA	NA
P4	+ve	-ve	Rod	NA	Catalase – ve	NA	NA	Acid +ve Gas –ve	+ve	NA	NA
P5	+ve	-ve	Rod	NA	Catalase – ve	NA	NA	Acid +ve Gas +ve	NA	NA	NA
P6	+ve	-ve	Rod	NA	Catalase – ve	NA	NA	Acid +ve Gas +ve	NA	NA	NA
P7	+ve	-ve	Rod	NA	Catalase – ve	NA	NA	Acid +ve Gas –ve	+ve	NA	NA
P8	+ve	-ve	Rod	NA	Catalase – ve	NA	NA	Acid +ve Gas +ve	NA	NA	NA
P9	+ve	-ve	Rod	NA	Catalase – ve	NA	NA	Acid +ve Gas +ve	NA	NA	NA
P10	+ve	+ve	Rod	Starch -ve	Catalase – ve	Cit -ve	+ve	NA	NA	NA	NA
P11	+ve	+ve	Rod	Sratch +ve	Catalase – ve	Cit -ve	+ve	NA	NA	NA	NA
P12	+ve	+ve	Rod	Starch -ve	Catalase – ve	Cit -ve	+ve	NA	NA	NA	NA
P13	+ve	-ve	Rod		Catalase – ve	NA		Acid +ve Gas +ve	NA	NA	NA

Inference: The above table shows the identification tests performed for 13 varieties of bacteria and their biochemical characteristics.

Table 2: List of all the bacteria isolated and identified in the study

Pure culture	Name of the bacteria
P1	Bacillus popilliae
P2	Bacillus cirulans
P3	Bacillus popilliae
P4	Lactobacillus casei
P5	Lactobacillus fermentii
P6	Lactobacillus fermentii
P7	Lactobacillus casei
P8	Lactobacillus casei
P9	Lactobacillus casei
P10	Bacillus larvae
P11	Bacillus larvae
P12	Bacillus larvae
P13	Lactobacillus fermentii

Inference: The above table shows varied group of microbial growth in the formulated media.

Conclusion

Study aimed at formulation of a nutritional media for the growth of microorganisms and also the best utilization of organic Kitchen and temple waste in laboratory procedures.This proposed work is not only important for laboratory media production but also add on to the waste disposal problem and is environment friendly. The only suspected aspect was the efficacy of the media in allowing the growth of varieties of microbial cultures. However the media was proved to be highly efficient in encouraging the growth of all kinds of bacteria and additionally the kitchen waste media showed contamination with fungi after a long term storage of 4 to 5 days indicating that it is efficient for the isolation of fungal strains also. The media can be formulated as per the users requirement and prepared with minute alterations like eliminating the use of agar for broth cultures, addition of antibiotics and changed pH for the isolation of fungal cultures etc. All the results obtained proved the efficacy of these two samples in promoting the growth of ubiquitous microbes. Further the work was extended towards the identification of all the bacterial species obtained which revealed a wide varieties of bacteria especially the species of lactobacillus were predominant in the isolates. Thus this can be an efficient method for solid waste disposal.

References

1) Ahmed A, Dadi L, Jida M, Emana M (2021) Studies on Utilizations of Floral Waste by Conversion to Value Added Products. Environ Pollut Climate Change. 5: 251

2) Avery RC, Leland SJ. A SIMPLE METHOD FOR THE ISOLATION OF PURE CULTURES FROM SINGLE BACTERIAL CELLS. J Exp Med. 1927 May 31;45(6):1003-7. doi: 10.1084/jem.45.6.1003. PMID: 19869299; PMCID: PMC2131162.

3) Bonnet M, Lagier JC, Raoult D, Khelaifia S. Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. New Microbes New Infect. 2019 Nov 30;34:100622. doi: 10.1016/j.nmni.2019.100622. PMID: 31956419; PMCID: PMC6961714.

4) Ferronato N, Torretta V. Waste Mismanagement in Developing Countries: A Review of Global Issues. Int J Environ Res Public Health. 2019 Mar 24;16(6):1060. doi: 10.3390/ijerph16061060. PMID: 30909625; PMCID: PMC6466021.

5) Lapage SP. Biochemical Tests for Identification of Medical Bacteria. J Clin Pathol. 1976 Oct;29(10):958. PMCID: PMC476234.

6) Rastogi M, Nandal M, Khosla B. Microbes as vital additives for solid waste composting. Heliyon. 2020 Feb 19;6(2):e03343. doi: 10.1016/j.heliyon.2020.e03343. PMID: 32095647; PMCID: PMC7033521.

7) Roslan MAM, Jefri NQUA, Ramlee N, Rahman NAA, Chong NHH, Bunawan H, Bharudin I, Kadir MHA, Mohammad M, Razali H. Enhancing food waste biodegradation rate in a food waste biodigester with the synergistic action of hydrolase-producing Bacillus paralicheniformis GRA2 and Bacillus velezensis TAP5 co-culture inoculation. Saudi J Biol Sci. 2021 May;28(5):3001-3012. doi: 10.1016/j.sjbs.2021.02.041. Epub 2021 Feb 18. PMID: 34012331; PMCID: PMC8117001.

8) Sanders ER. Aseptic laboratory techniques: plating methods. J Vis Exp. 2012 May 11;(63):e3064. doi: 10.3791/3064. PMID: 22617405; PMCID: PMC4846335.

9) Tripathi N, Sapra A. Gram Staining. 2021 Aug 11. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 32965827.

10) Kwoji ID, Okpeku M, Adeleke MA, Aiyegoro OA. Formulation of Chemically Defined Media and Growth Evaluation of *Liqilactobacillus salivarius* ZJ614 and *Limosilactobacillus reuteri* ZJ625. Front Microbiol. 2022 May 6;13:865493. doi: 10.3389/fmicb.2022.865493. PMID: 35602032; PMCID: PMC9121020.