“A NOVEL BACTERIAL CULTURE MEDIA”

Dissertation Submitted to the
Dr. Babasaheb Ambedkar Technological University, lonere

In Partial fulfillment of
the requirements for the degree of

BACHELOR OF PHARMACY

In

PHARMACY

By
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It is to certify that ***Mr. Rahulsing Govindsing Girase*** ***(Seat no.: 21542820181382310022)*** studying in the Eightth Semester of Bachelor of Pharmacy, has attended the viva-voce and defended the dissertation/thesis entitled **"A Novel Bacterial Culture Media"** at Ahinsa Institute of Pharmacy, Dondaicha.

Sign of the External Examiner
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This is to certify that this dissertation entitled **"A Novel Bacterial Culture Media"** is a Bonafide and genuine research work carried out by **Mr. Rahulsing Govindsing Girase** at Department of pharmacy, Ahinsa Institute of Pharmacy, Dondaicha.in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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DECLARATION BY THE CANDIDATE

This is to certify that this dissertation entitled **"A Novel Bacterial Culture Media"** is a bonafide and genuine research work carried out by me under the guidance of **Miss. Neha R. Jaiswal** Assistant Professor, Department of Pharmacy at Ahinsa Institute of Pharmacy, Dondaicha.

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ENDORSEMENT BY THE HOD/ PRINCIPAL

This is to certify that this dissertation entitled **"A Novel Bacterial Culture Media "** is a bonafide and genuine research work carried out by **Mr. Rahulsing Govindsing Girase** under the guidance of **Miss. Neha R. Jaiswal** , Assistant Professor, Department of Pharmacy at Ahinsa Institute of Pharmacy, Dondaicha.

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Date:

AKNOWLEDGMENT

"This is an effort not only by me but my Family and friends, a dream visualized by them to see me excel".

The task of preparing the dissertation has been a fascinating experience and it is really a moment of great pleasure for me to express my hearty gratitude to those who have helped me in the successful completion of this dissertation.

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*I owe the success of this work to my **"Family"** who always have been the source of inspiration and without whose support this project could never been accomplished.*

Name of Candidates

Mr. Rahulsing Govindsing Girase

Affectionately Dedicated To

My Family

Whose love and affection are infinite

My Friends

Who are always with me in adversity and prosperity

My Guide

To whom I shall remain indebted for giving new shape and path to my life

My God

ABSTRACT

The development of microbiology began in the 19th century with the invention of the culture medium. Bacterial culture was the first method to study human microbiota in 1860. Louis Pasteur was the first to propagate bacteria on Culture media. The culture media provides the essential nutrients such as carbohydrates, protein, vitamins & some growth factors for the proper development of bacteria. Nowadays, these growth medias are prepared by different expensive chemical ingredients in laboratories for research experiments, which ultimately makes our experiments expensive.

Instead of using high-cost culture media, fruit waste material could prove to be a good alternate Source for the production of low-cost media. The waste generated in household practices & kitchen includes vegetables & Fruit waste. It is waste that almost every house generates every day. It can serve as a good source of nutrients & Vitamins for in microorganisms. Hence these materials can be used to formulate solid media for the growth of bacteria. In the current study waste material like Mango peels, Banana peels, Lemon peels & Ground nut shell have been included to formulate the media. This material was collected from kitchen waste & vegetable market. Comparing the growth of organism (*Lactobacillus bulgaricus*) with standard Commercial media and fruit waste with agar, it was found that the media prepared from Fruit waste serves as a good and inexpensive source of nutrients for many bacteria. Thus, it can be good further and used Commercially for isolation and cultivation of various microorganisms.

Keywords: Culture media, Bacteria, Fruit waste, Nutrients, *Lactobacillus bulgaricus.*

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CHAPTER 1: INTRODUCTION

1. INTRODUCTION

A microorganism requires nutrients, a source of energy and certain environmental Conditions for their proper growth and reproduction. These requirements are mainly met by Culture Medium in laboratories. When medium is being prepared for microbial growth consideration must be given to Carbon and energy sources and other growth factors that essential for the organisms. [1]

Culture media is of prime importance in field of microbiology and applied sciences. Any medium in its liquid or solid State is used to grow, enrich, observe and quantify microorganism on solid medium One can observe the particular colony characteristics shown by an organism while in liquid form organism can be enriched to obtain excess yield various of the microbial product. Nowadays, various Commercial media ore available for growth of specific type of microorganism which are more costly to afford, To Find the cheap and cost effective medium, scientist continuously searching way to design a media.[2]

The first liquid artificial culture medium was prepared by louis Pasteur in 1860, which revolutionized the microbiology. Previously growth of bacteria was observed on materials such as some foods. These observations highlighted the importance of bacteria's natural environment and their nutritional needs in the development of culture media. A culture medium essentially composed of basic elements (water, nutrients),[3] macro elements like C, O, H, N, S, P, K, Ca, Mg, and Fe out of this element first six are used in the synthesis of carbohydrates, lipids, proteins and nucleic acids and remaining elements present in the cell as cations which performed different roles.[4]

1.2 TYPES OF CULTURE MEDIA [5]:

The culture Medias are classified in many ways-

1) Based on physical state-

A) Liquid media

B) Semi solid media

C) Solid media

2) On the basis of presence of molecular oxygen and reducing substances in the media.

A) Aerobic media

B) Anaerobic media

3) Based on Nutritional factors-

A) Simple media

B) Complex media

C) Synthetic media

D) Special media.

1) Based on physical state:

Bacteriological culture media can be prepared in liquid (broth), a solid (plate media or slant media) or semi-solid state as shown figure. Solid and semi-solid media contain a solidifying agent such as agar or gelatin, agar is a polysaccharide which is extracted from seaweed (Rhodophyceae) is used because of its inert and non-nutritive nature.

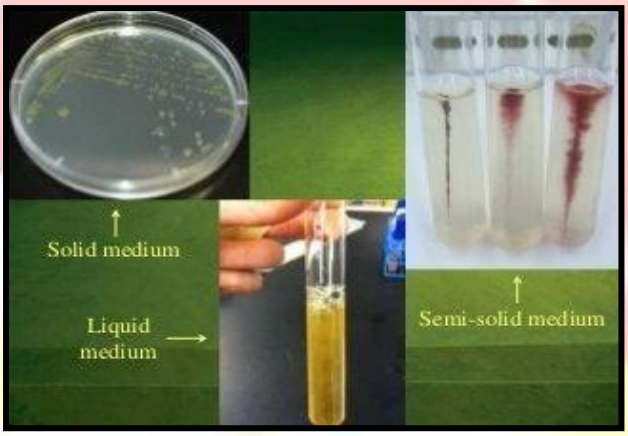


Fig.1.1 Solid, liquid and Semisolid media[29]

2) Based on the presence of molecular Oxygen & reducing substances in the media:

A) Anaerobic media:

These are utilized for the cultivation of anaerobic bacteria eg.

a) cooked meat broth (CMB)

b) Thioglycollate broth.

Fig. 1.2 Cooked meat broth[30]

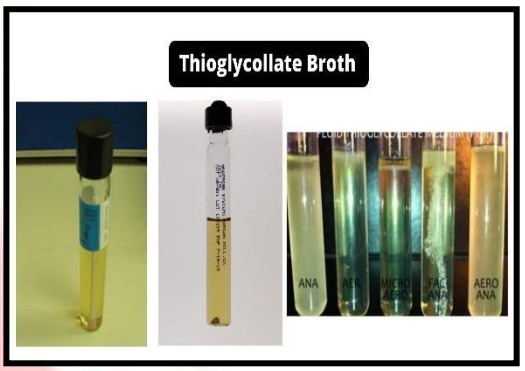


Fig. 1.3 Thioglycollate broth[31]

B) Media for Testing special properties:

Urea medium is used to test the property of urease production phenyl pyruvic acid is employed for identification of proteus sp.

3) Based on nutritional factors:

A) Simple media:

The example of simple media is nutrient broth which contains peptone water and meat extract 1%. To this medium when 0.5% glucose is added it becomes glucose broth as shown in fig.When 2-3% agar is added to nutrient broth it becomes nutrient agar. If concentration of agar is reduced to 0.2 to 0.4% Semisolid medium is obtained which allows the motile bacteria to spread.

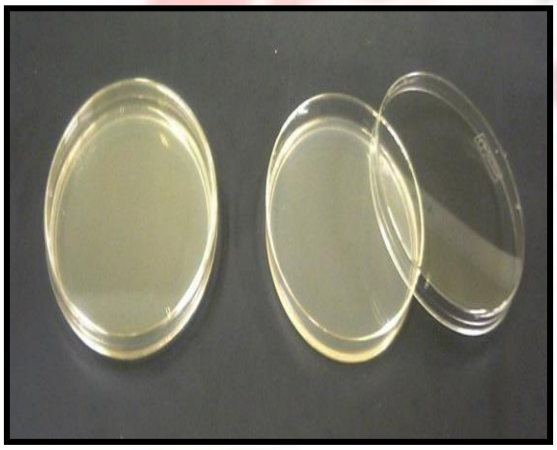


Fig.1.4 Nutrient agar[25]

Fig. 1.5 Nutrient broth[36]

B) Complex media:

All media other than simple media are complex. This media includes ingredients for bringing out certain property of providing special nutrients required for the growth of the bacteria in question.

C) Synthetic media:

These types of media are synthesized from pure chemicals and composition of medium also known. These are used for Special studies such as metabolic requirements. Dubo's medium with tween 80 is one example of synthetic medium.

D) Special media:

a) Enriched media:

When basal medium is mix with some nutrients such as blood, serum or egg it is called enriched medium eg. Blood agar, chocolate agar and Loeffier's serum slope.

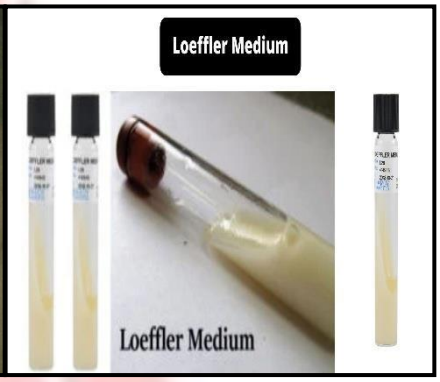


Fig. 1.6 Blood agar[26] **Fig. 1.7 Chocolate agar[27]** **Fig. 1.8 Loeffiers serum slope[28]**

b) Enrichment media:

Some Substances are incorporated in the liquid medium which have stimulating effect on the bacteria to be grown or inhibits its competitors eg. Tetrathionate broth, Selenite 'F' broth and Alkali peptone water.

Fig. 1.9 Selenite F broth, Tetrathionate broth and Alkaline peptone water.[29]

c) Selective media:

It contains media Substances that inhibit all but few types of bacteria & facilitate the isolation a particular species. This media is used to isolate particular bacteria from specimen where mixed bacterial flora is expected. Selective media are solid in contrast to enrichment media which are liquid eg. Deoxycholate Citrate agar, bile salt agar (BSA).

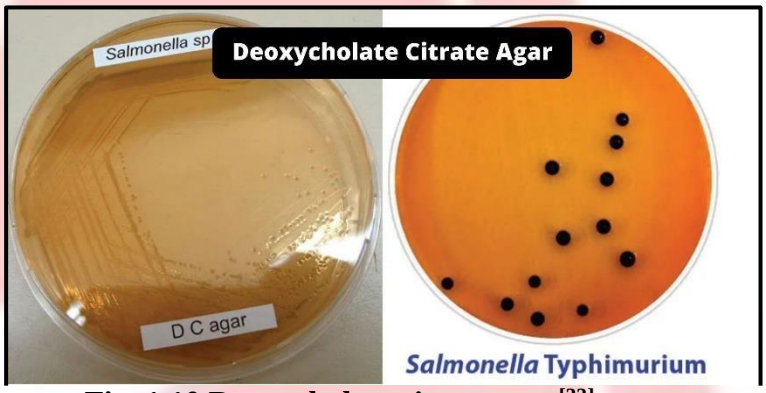


Fig. 1.10 Deoxycholate citrate agar[33]

D) Differential Media:

When a medium contains Substances which help to distinguish differing characteristics of bacteria, it is called differential medium eg. Macconkey's medium.

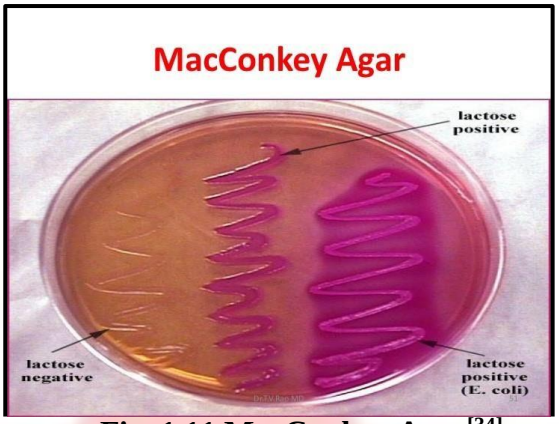


Fig. 1.11 MacConkey Agar[34]

e) Indicator media:

These media contain an indicator which changes color when a bacterium grows in them Salmonella typhi grown as black colonies on Wilson and Blair medium containing sulphite eg. Macconkey's Agar.

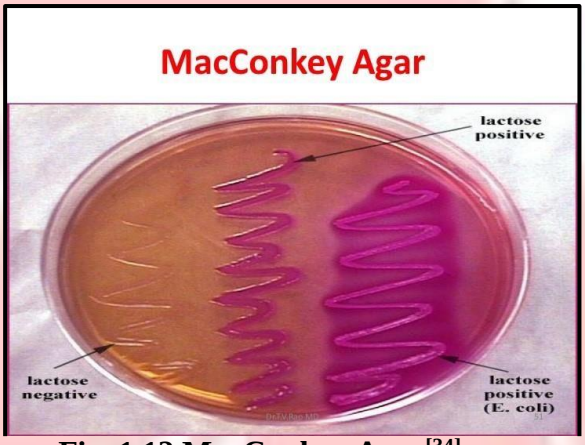


Fig. 1.12 MacConkey Agar[34]

f) Transport media:

These are used in the case of delicate organisms (eg. Gonococci) which may not survive the time taken for transit or may be overgrown by nonpathogenic bacteria (eg. Cholera organisms). For transport of specimens to the laboratory, special media devised and these are termed ‘transport media’. Eg. Transport medium and Buffered glycerol Saline transport media.

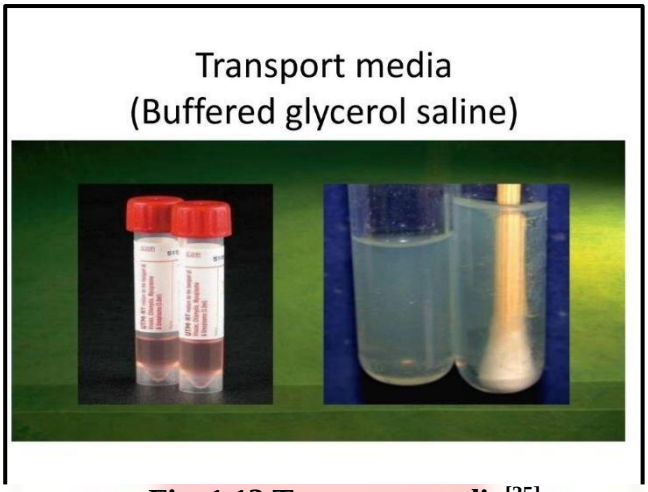


Fig. 1.13 Transport media[35]

g) Sugar media:

This media help in identification of bacteria. The term sugar in microbiology denotes any fermentable substance.

Glucose, lactose, sucrose & mannitol are routinely employed for fermentation tests. It contains. 1% Sugar in peptone water along with an Indicator (Andrade's indicator).

1.3 Nutritional requirements of bacteria[6]:

In order to grow well, bacteria need at least nutrients such as water, a carbon source, a nitrogen source and mineral salts.

1) Water:

water plays vital role in solubilizing nutrients, transporting them and ensuring hydrolysis reactions. Some bacteria require. Free water for their growth.

2) Carbon sources:

Carbon is main constituent element in bacteria, it is essential for the synthesis of molecules such as fats, carbohydrates, proteins and nucleic acids. Bacteria can get carbon from inorganic carbon sources, such as carbon dioxide or organic sources such as sugars and alcohols.

3) Nitrogen sources:

they are numerous and can be found in a large number of compositions of culture medium. It is found the in organic form, corresponding protein hydrolysates. Nitrogen allows bacteria to

synthesize their proteins. Finally, among the common mineral salts, phosphate, sulphate, magnesium or calcium are regularly found.

4) Growth factors or bacterial vitamins:

Some species of bacteria requires organic compounds in minimum amount for growth which is called as growth factors or bacterial vitamins. Some bacteria have ability to synthesize their entire requirement of vitamins from culture medium. Some species cannot synthesize the vitamins from media and don't show growth in the absence of vitamins so, they are must be added in media. [7] Nutrient agar (NA) is commonly used as general-purpose medium for the growth of a cultivation of broad range of bacteria. It is mainly composed of peptic digest of animal tissue, beef extract and yeast extract, sodium chloride & agar. Commercially available media such as nutrient agar (na), cetrimide agar macconkey agar are also used for the growth of microorganisms but they are very expensive.

in this research project we are developing alternative and cost-effective culture media using fruit waste like, mango peels, banana peels, lemon peels & ground shell. This waste materials were procured from household waste and local market. Which is further processed and used in the formulation of culture media. Such waste is rich in carbohydrates, proteins and other nutrients which are easily utilized by the microorganisms. The nutrient agar (na) is used in this project as standard media to compare the growth of formulated media

1.4 Isolation methods for pure cultures[23]:

1) Streak plate method:

the bacterial cells are deposited on media by the establishment of gradient across the face of the petri plate. This method is utilized to isolate the pure cultures of bacteria. Small sample of test culture is placed and streaked across the plate surface with the help of inoculation loop and the plates placed for the incubation.

fig.1.14 Streak plate method[37]

2) Pour plate method:

in this technique, the mixed culture of bacterium is diluted directly within the liquid agar medium tube (42-45°c) and mixed well. During this technique, mounted quantity of inoculant (generally one ml) from a broth/sample is placed within the center of sterile petri dish employing

a sterile measuring device. The contents of every tube area unit poured into separate petri plates and so allowed to solidify. Finally, isolated the colonies and area unit picked up by immunization loop and patterned onto another petri plate to insure purity. This technique is additionally used for determinative the quantity of viable microorganism cells.

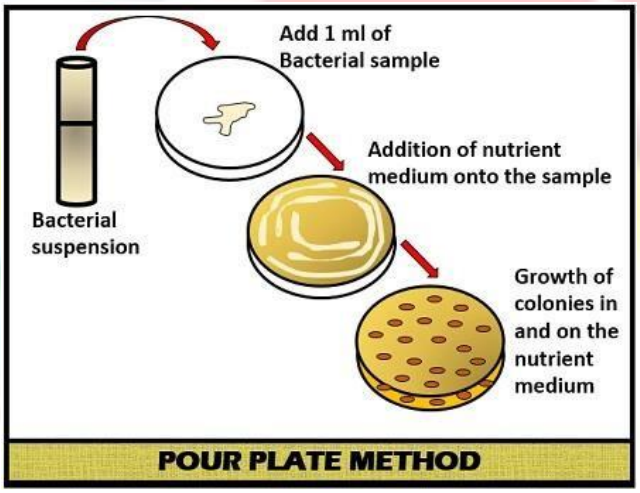


fig. 1.15 Pour plate method[38]

3) Spread plate method:

The principle of this technique involves employing a sterilized spreader with a sleek surface product of metal or glass to use a tiny low quantity of microorganism suspended in an exceeding resolution over a plate that must be dry and at temperature in order that the agar will absorb the Microorganism a lot of pronto. A productive unfold plate can have enumerable range of isolated microorganism colonies equally distributed on the plate.

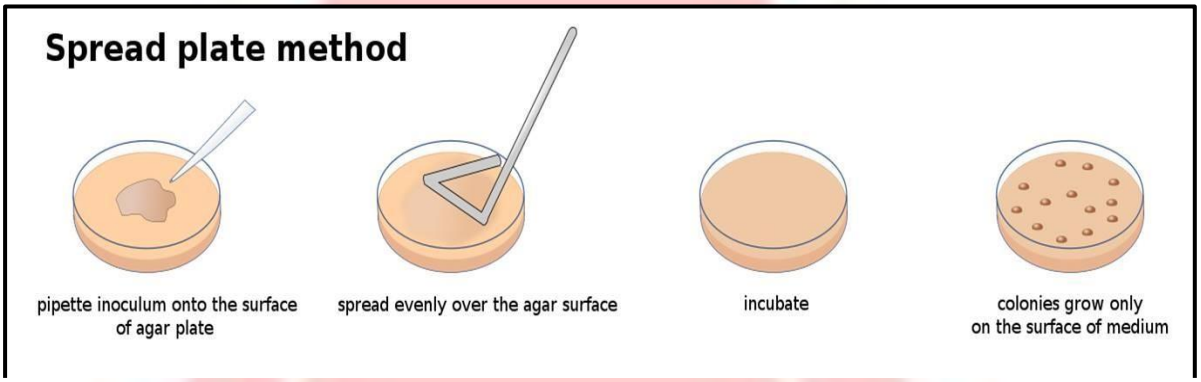


Fig. 1.16 Spread plate method[39]

1.4 STERILIZATION METHOD OF CULTURE MEDIA AND APPARATUS[40]:

The culture media and apparatus are generally sterilized by **Autoclave method** which is a sealed device (similar to a pressure cooker) that kills microorganisms.

Principle:

The use of moist heat facilitates the killing of all microorganisms, including heat-resistant endospores which is achieved by heating the materials inside the device at temperatures above the boiling point of water.

The usual procedure is to heat at 1.1 kilograms/square centimetre (kg/cm2) [15 pounds/square inch (lb/in2)] steam pressure, which yields a temperature of 121°C. At 121°C, the time of autoclaving to achieve sterilization is generally considered to be 15-20 min, depending on the volume of the load.

To make sure, sterilization is successful one should ensure:

1) Air should be evacuated so that the chamber fills with steam.
2) Articles should be placed in the autoclave so that steam can easily penetrate them.

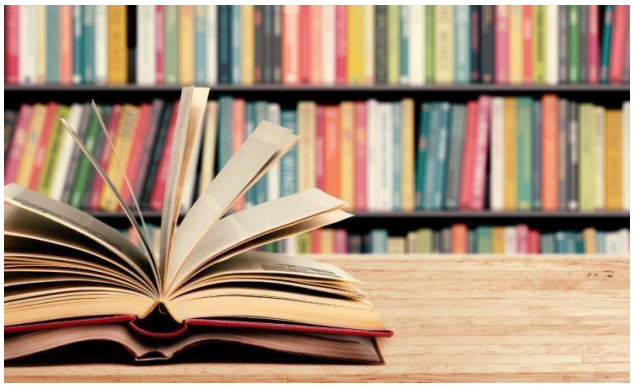
Fig. 1.17 Autoclave

1.6 ADVANTAGES OF CULTURE MEDIA:

1) It is useful for the growth of bacteria.
2) It is cost-effective than commercial media's
3) The growth of microorganisms is better than commercial available media at low coast.
4) As it is prepared from waste material, It can be overcome the problem of waste management.
5) It is environmental friendly.

1.7 DISADVANTAGES OF CULTURE MEDIA:

1) Risk of contamination.
2) It requires high level of sterilization.
3) Chances of getting infection with pathogenic microorganisms.



CHAPTER 2: LITERATURE REVIEW

2. LITERATURE REVIEW

M. Bornet et.al…..(2020) Microorganisms requires basic nutrients, a source of energy and environmental conditions in order to grow and reproduce. microorganism adopt habitats most suitable for their growth in the environment, to provide these requirements of microbes a 'culture medium' was discovered by Louis Pasteur in 1860.

Pratibha Jadhav et.al…. (2017) To prepare medium for microorganism growth, The supply of carbon and energy sources and other growth factors is essential for the organisms. Depending on the nutritional growth requirements of microorganism, culture media are of different types. The microorganism requires different macro elements likely C, H, 0, N, S, P, K, Ca, Mg and Fe. for the synthesis of proteins, lipids, Carbohydrates, Nucleic acids. C, H, O, N, S & P are used. while K, Ca, Mg & fe act as cell cations other elements like Mn, Zn, CO, MO, Ni & Cu these are the part of enzymes & cofactors.

Sayali Daptardar et.al….(2018) Growth medium in it 's liquid state or solid state can be used to grow, enrich, observe and quantity microorganisms. Solid media can show the extraordinary colony chracteristics Shown by an organism while liquid medium can be used to obtain the high yield of enriched microorganism.

John Linduist et.al….(2006) A numerous type of Culture media are utilized by the microbiologist to isolate, maintain grow the pure cultures and identify the bacteria according to their biochemical and physiological characteristics

HiMedia…. Nutrient agar medium is frequently used as general-purpose medium for cultivation of wide range of bacteria. It is basically composed of peptone, meat extract, Beef extra, sodium chloride and agar.

Pratibha Jadhav et.al….(2017) Commercially available media such, Nutrient Agar (NA), Centrimide agar, MacConkey Agar are used for the growth of microorganisms but these are very expensive.

Scientists are making efforts continuosiy for finding an alternative source and culture media to design the cost-effective medium that can facilitate the growth of microorganism.

Sunil Kumar et.al...(2017) Waste management is a problem all over the world. Especially in developing countries like, India, increasing waste and managing its disposal is a big problem. 170000 tonnes of waste are generated in urban areas of India every day, 40- 60% of waste emanating in urban through household practices.

Issac o. Et. Al…… (2014) This waste can be classified into two distinct forms like Dry waste and wet waste. Wet waste generally contains organic waste. 2-3 thousand tonne of organic Kitchen waste are generated per year which can become an effective resource for recovery of recyclable materials. wet organic waste emanating from kitchen and household practices includes fruits and vegetable wastes like peels, pulp, seed, pomace, stems, pods etc. Such waste is rich in carbohydrates & other nutrients which are used by microorganism n alternative source for the cultivation microorganisms

Saheed OK et.al….(2013) The fruit wastes such as mango peel and ground nut shell can be a good source of nutrients of nutrients for the growth of bacteria as it contains simple and complex sugars that are metabolize by microorganisms**.** It also contains other nutrients such as protein, fibre, carbohydrate & ions like sodium, potassium, magnesium, calcium, iron, zinc and phosphorus etc.

Deivanayaki M. et. Al……(2012) The increasing cost of culture media has necessitated continuous search for replacing the commercial media with cheap alternative media. considering the commercial media 100 gm of Nutrient Agar powder cost approximately 330/- in India (Hi Media Laboratories) Mumbai) Thus 1kg of this media costs around 3,300/-

The problem of waste management can be overcome by utilizing the waste at large scale for the production of culture media. This can also minimize the problem of costly commercial media by using natural media prepared by using the fruit waste and vegetable waste. Standard procedures in any microbiological laboratories require large number 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.

CHAPTER 3: AIM AND OBJECTIVES

3. AIM AND OBJECTIVES

AIM:

- Formulation of culture media for the growth of bacteria using fruit waste.
- To grow test bacteria on formulated fruit waste agar.
- Analysis of cost-effectiveness of fruit waste agar.

OBJECTIVES:

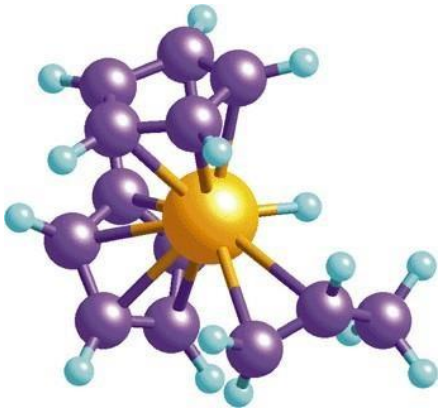
- The main underlined objective is to make cost effective culture medium for bacteria which can be easily utilized by the bacteria for their growth.
- To recycle the waste material emanating from kitchen.
- Isolation of test bacteria on formulated culture media.
- To determine various characteristics of powder such as organoleptic, particle size, bulk density, flow property, pH, solubility etc.
- To overcome the major problem of domestic waste, kitchen waste through utilising the waste.

CHAPTER 4: PLAN OF WORK

4. PLAN OF WORK

The plan of work was planned as per the following experimental protocol.

- Literature survey.
- Selection of topic.
- Selection of material.
- Collection of material.
- Processing of material.
- Drying of selected material.
- Formation of powder.
- Determination of powder characteristics (Organoleptic, Bulk density, Particle size, Flow property, pH, Solubility etc.)
- Selection of extraction method.
- Extraction of powder.
- Selection of test organism.
- Selection of Inoculation method.
- Formulation of culture media.
- Isolation of bacteria on formulated media as well as Standard commercial media.
- Incubation of culture media.
- Observation of results.
- Results and discussion.
- Conclusion.
- Printing and binding of thesis



CHAPTER 5: MATERIAL PROFILE

5. MATERIAL PROFILE

1) Mango peel powder:[14,15]

Fig. 5.1 Mango peel powder.

Genus: Mangifera.

Biological Source: A mango is an edible stone fruit obtain from Mangifera indica plant.

Family: Anacardiaceae.

Synonym: Mangifera indica.

Nutrients present- Mango peel contains a variety of macronutrients (total carbohydrates (20-30%), protein, amino acids, lipids, organic acids as well as dietary fiber) and micronutrients. Dietary Fiber is important functional nutrient it ranges between 16% to 28%. The content of Vitamin C ranges from 188-2570 ug/g. The mango peel contains significantly higher levels of minerals than pulp of the following minerals Ca, k, Mg, Na, fe, Mn, Zn, Cu.

2) Banana peel powder:[16,17]

Fig. 5.2 Banana peel powder.

Genus: Musa.

Family: Musaceae.

Biological Source: A banana is an elongated, edible fruit botanically berry-produced by Several kinds of large herbaceous flowering plant.

Nutrients: Significant quantities of banana peels, equivalent to 40% of the total weight of fresh banana are generated as waste. It contains Following nutrients per 100 gm, protein 7.57 ± 0.30 gm 10.0% Fiber, 68.39 carbohydrate & macronutrients like Na 0.18 gm/100 gm, 9.39 gm/100 gm potassium 0.71 gm/100 gm Magnesium, 0.44 ± 0.08 g/ants in the genus Musa.

Synonym: Musa coccinea, Musa velutina. 100mI Calcium, 96.50 mg/kg iron, 27.95 mg/kg Zinc, 0.09 gm/100 gm phosphorus.

3) Groundnut shell powder:[18,19]

Fig. 5.3 Ground nut shell powder.

Genus: Arachis.

Biological Source: It obtain from the plants Faboideae, Subfamily of the legumes which grow &ripen underground.

Family: Fabaceae

Synonyms: Peanut, goober, Pindar.

Nutrients: It includes 0.50 % Crude protein. 59.0 % crude fiber, 4.43% carbohydrate. Sodium (42.00 mg/100 gm) potassium (705.11 mg/100 gm), Magnesium (3.98.00 mg/100 gm) Calcium (2.28 mg / 100 gm), iron (6.97 mg / 100gm), zinc (3.20 mg/100 gm), phosphorus (10.55 mg/100 gm) are all abundant in groundnut shells.

4) Lemon peel powder:[20,21,22]

Fig. 5.4 Lemon peel powder.

Genus: Citrus.

Biological Source: The lemon (citrus limon) is a Species of small evergreen trees in the flowering plant family Rutaceae.

Family: Rutaceae

Synonyms: Citrus limon, citrus aurantium subsp. Bergamia.

Nutrients: The lemon peel contains the protein (1.5 gm/100 gm), Fibre (11 gm/ 100 gm), Carbohydrate (16 gm/100 gm) and macro elements, sodium. (1.99 mg/100g), potassium (127 mg / 100 gm), magnesium (11.50 mg / 100 gm), calcium (31.8 mg / 100 gm), iron (0.34 mg/100 gm), zinc (0.01 mg / 100 gm), phosphorus (23. 9 mg / 100 gm). It includes other growth factors like, Vitamin A and E, Vitamin B.

5) Sodium chloride (Nacl):[24]

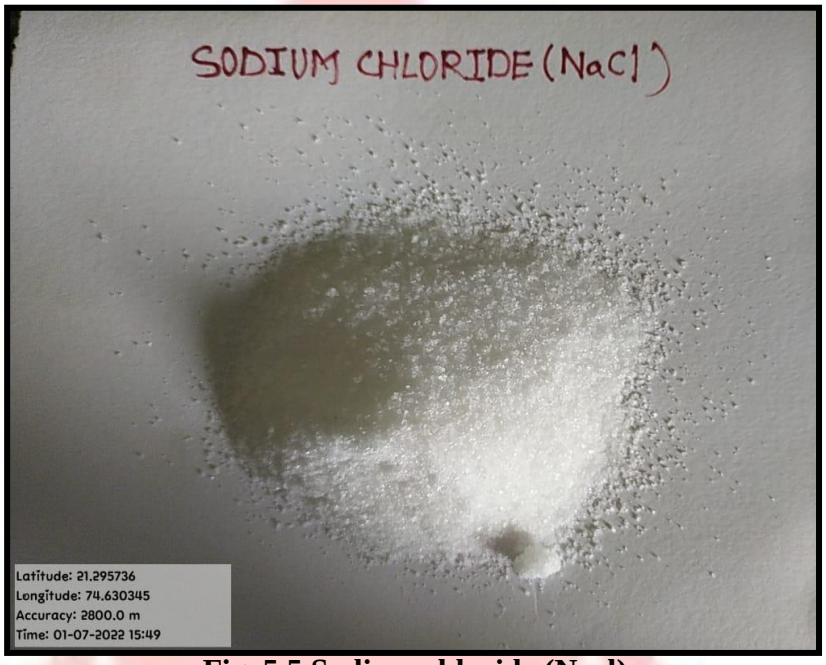


Fig. 5.5 Sodium chloride (Nacl)

Sodium chloride is the most well-known salt and consists of a single sodium ion that is bonded to a single chlorine ion. The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is Similar to the cytoplasm of microorganisms. If the Salt concentration is not similar osmosis takes place transporting excess water into or out from the cell. Both of these scenarios can be led to the death of the cell.

6) Agar:[24]

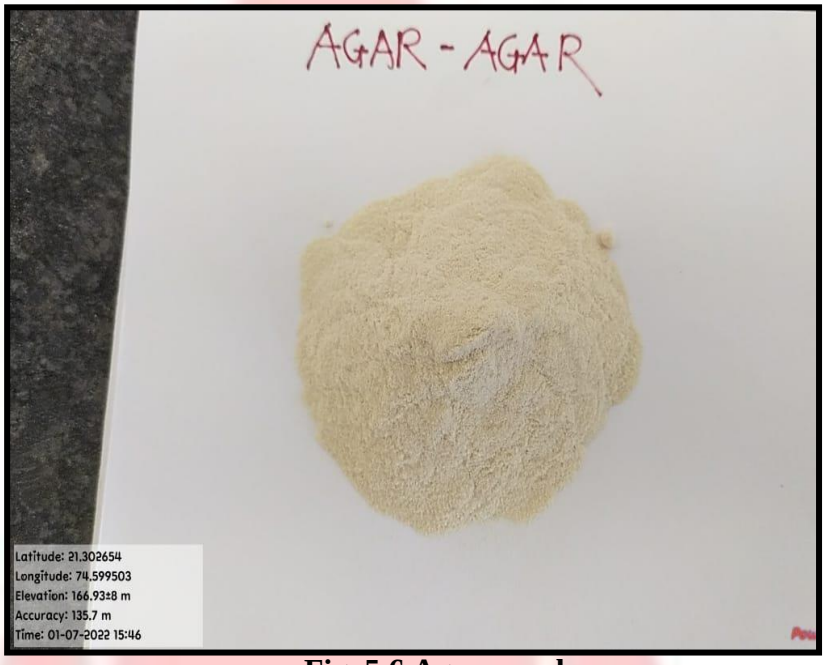


Fig. 5.6 Agar powder

A large proportion of culture media are consist of the chemical agar. Agar is a gelatinous mixture that is extracted from seaweed. Agar consists of mixture of polymers (polysaccharides), where the basic sugar is galactose and it is used to solidify the medium.

7) water:

water makes up a large proportion of media. water is essential for the growth and reproduction of microorganisms and also provides the medium through which various nutrients can be transported.

CHAPTER 6: MATERIAL AND METHODOLOGY

6. MATERIALS AND METHODS

6.1 SELECTION OF RAW MATERIALS:

The materials for a formulation were selected on the basis study of different brief literature review and surfing of general and publication.

6.2 COLLECTION OF RAW MATERIALS:

The waste materials such as mango peels, banana peels, lemon peels and ground but shell were procured from kitchen waste and vegetable market. One kilogram of each waste material was collected. Samples were brought to the laboratory using sterile plastic bags for further processing.

6.3 PROCESSING OF WASTE MATERIAL:

The waste of fruits was 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.

6.4 FORMATION OF POWDER:

The raw material was sun dried for 3-4 days. Dried raw materials were ground by using electronic blender to obtain its powdered form. Each powder was sieved through sieve no. #100 to Obtain finer particles of the powder and then stored in clean and dry plastic containers.

6.5 TEST ORGANISM USED: *Lactobacillus bulgaricus*

6.6 FORMULATION OF MEDIA: -

1) One gram powder of each ingredient was added in four different clean and sterile conical flask (100 ml) containing 20 ml warm distilled water, which was plugged with cotton and kept overnight for obtaining its natural extract.
2) Each solution was then filtered with filter paper.
3) 5 ml of each filtrate was mixed one by one in conical flask.
4) To this 2.5 gm of sodium chloride was added.

5) 3% Agar added to the solution with stirring without forming clumps for solidification.
6) The volume was made up to 100 ml with distilled water.
7) pH was maintained at 7 ± 0.5.
8) Media was autoclaved and poured into sterile petri plates.

6.7 FORMULA TABLE FOR 100 ML: -

Sr. No	IGREDIENTS	F1	F2
1.	Mango Peel Extract	5 ml	5 ml
2.	Banana Peel Extract	5 ml	5 ml
3.	Lemon Peel Extract	5 ml	5 ml
4.	Groundnut Peel Extract	5 ml	5 ml
5.	Sodium Chloride (Nacl)	2.5 gm	2.5 gm
6.	Agar	3 gm	2 gm
7.	Water	Q. S	Q. S

Fig. 6.1 Extraction of powder

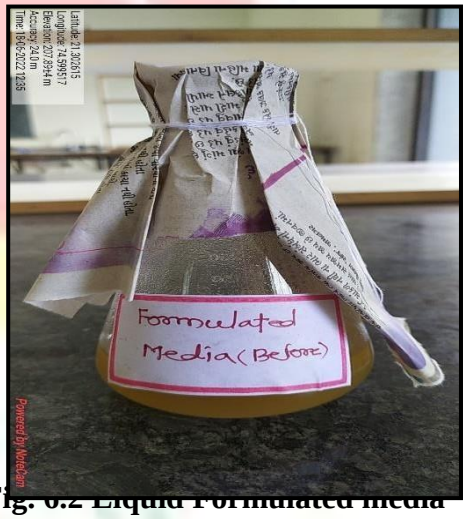


Fig. 6.2 Liquid Formulated media

6.8 STERILIZATION OF MEDIA AND APPARATUS: -

The media and apparatus were sterilized by Autoclave method. This technique works on the principle of Moist heat sterilization. It kills the microorganisms by the irreversible denaturation of enzymes and structural proteins. The media and apparatus were wrapped with the paper and placed in autoclave for sterilization at 121°C for 20 minutes under 15 psi pressure and were poured into sterile petri dishes separately.

Fig. 6.3 Media and petri dish

Fig.6.4 Autoclave

6.9 ISOLATION OF BACTERIAL SPECIES: -

The lactobacillus bulgaricus bacteria was taken from curd sample. These bacteria were isolated on newly prepared fruit waste agar as well as standard commercial media Nutrient agar (Prepared by mixing the components obtained from HiMedia, Mumbai) using the streak plate method. The plates were incubated at 37°C for 24-48 hours in incubator. After the incubation these plates was observed and compared with standard Nutrient agar (NA) for bacterial growth.

CHAPTER 7: RESULTS AND DISCUSSION

7. RESULTS AND DISCUSSION

RESULTS:

1) Procurement, processing and preparation of powdered form of raw waste materials:

all the raw materials were procured and processed to obtain powdered form.

The fruit waste agar media was formulated by two formulaes (F1 and F2). Out of this two Formulae, the 'F1' formulae supported the growth of lactobasillus bacteria. Bacterial growth was better and requies less time to solidify on 'F1' formulation while 'F2' formulation require more time to solidify due to less concentration of agar. The bacterial growth was faster on fruit waste agar. The bacterial culture showed growth after 24 hours on this media while on Nutrient agar (NA) it took 48 hours to show the growth at 37℃. As shown in figure. Below, *Lactobasillus bulgaricus* bacteria showed luxuriant growth than the Nutrient agar (NA) media within 24 hours. Which later was confirmed on the electronic microscope at 45X power lens.

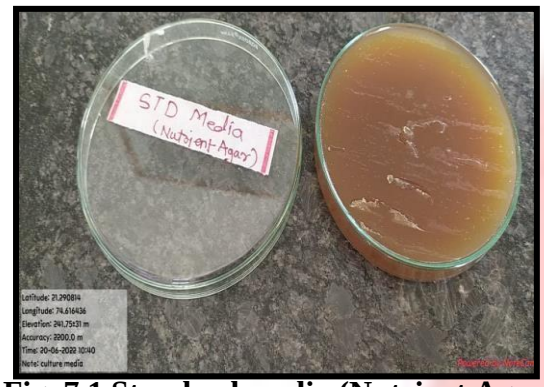


Fig. 7.1 Standard media (Nutrient Agar)

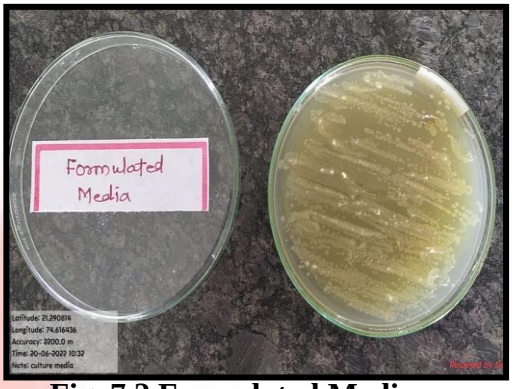


Fig. 7.2 Formulated Media.

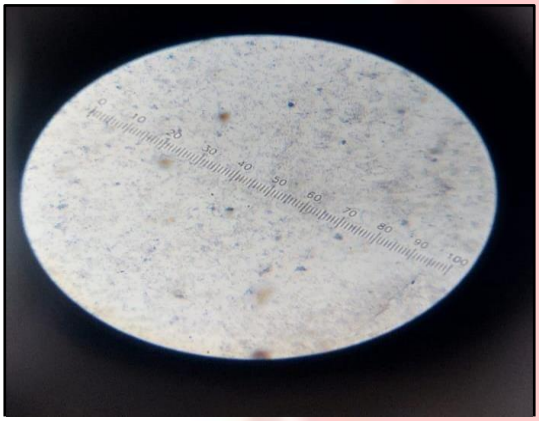


Fig. 7.3 Bacteria in Compound microscope.

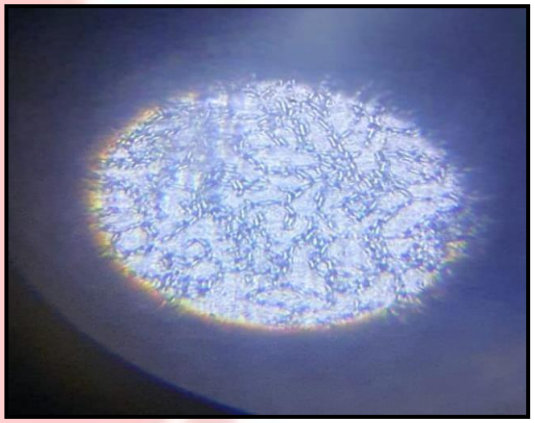


Fig. 7.4 Bacteria in Electron microscope

2) CHARACTERISTICS OF POWDER:

a) Organoleptic properties:

the organoleptic properties of mango peel powder, banana peel powder, lemon peel powder, ground nut shell powder was evaluated for their appearance, color, order, texture and smoothness shown in the below **Table 1.**

b) Particle size:

Particle size of mango peel powder, banana peel powder, lemon peel powder, ground nut shell powder was determined by sieving method with help of sieve shaker machine shown in the below **Table 1.**

c) Bulk density and Tapped density:

The bulk density and tapped density of mango peel powder, banana peel powder, lemon peel powder, groundnut shell powder was determined with the help of bulk density apparatus shown in the below **Table 1.**

d) Flow property:

The flow property of mango peel powder, banana peel powder, lemon peel powder, ground nut shell powder was determined by the method of angle of repose. Most of them showed excellent flow property shown in the below **Table 1.**

e) pH:

The pH of all ingredient powder was determined by pH paper method. The paper was dipped in solution of powder and color change compared with standard pH range shown in the below **Table 1.**

f) Solubility:

The solubility of powder was checked in water and all ingredients was soluble in water shown in the below **Table 1.**

Sr. No	Parameter	Mango peel	Banana peel	Groundnut shell	Lemon peel
1.	Appearance	Powder	Powder	Powder	Powder
2.	Color	Yellow	Brownish	Light Yellow	Pale Yellow
3.	Odour	Characteristics	Characteristics	Slight	Characteristics
4.	Texture	Moderate fine	Fine	Fine	Very Fine
5.	Smoothness	Smooth	Smooth	Smooth	Smooth
6.	Particle Size	180 ± 20 µm	150 ± 10 µm	150 ± 10 µm	150 ± 10 µm
7.	Bulk Density	0.52 gm/ml	0.40 gm/ml	0.21 gm/ml	0.49 gm/ml
8.	Tapped Density	0.60 gm/ml	0.57gm/ml	o.27 gm/ml	0.5 gm/ml
9.	Flow property (Angle of repose)	Excellent (24.22º)	Excellent (27.47º)	Excellent (25.64º)	Fair (36.50º)
10.	Ph	3-5	7-9	9-12	4-6
11.	Solubility	Water soluble	Water soluble	Water soluble	Water soluble

Table 1. Powder characteristics

DISCUSSION:

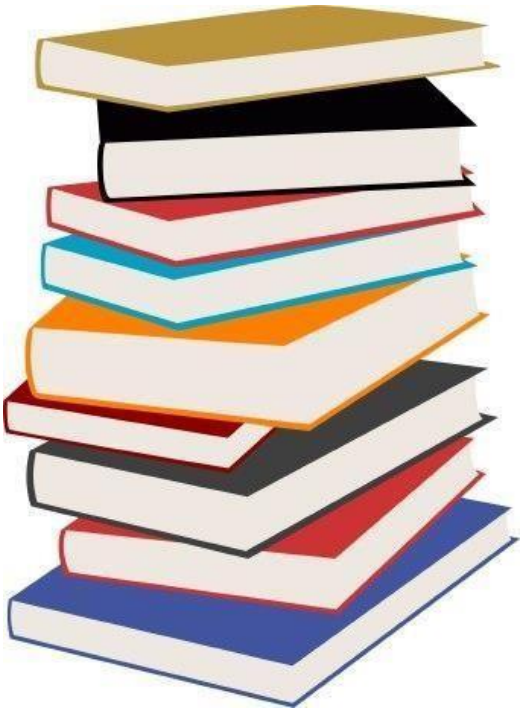
Cultivation of industrially important microorganisms requires large amount of growth media. Scientists have also concluded that the media formulated using organic waste can prove to be the replacement for the high- cost commercial media, *Deivanayaki et.al* formulated the media using the vegetables like tomato, carrot, cabbage and pumpkin. It supported the growth of bacteria and fungi in very less time as compared to commercially available growth media. *Pratibha Jadhav et.al* showed that fruit waste and vegetable waste like cauliflower stalk, potato peel, fenugreek stem and orange peel have been used in formulation of cheap alternative media which supported the growth of bacteria. *Sayali Daptardar et.al* used kitchen waste to formulate the media; it included pomegranate peels, sweet lime and orange pomace, peapods and spinach in the formulation of media for cultivation of fungi .

CHAPTER 8: CONCLUSION

8. CONCLUSION

Waste recycling is the best method of waste management. In the current study, fruit waste was used to formulate medium that can be used in every day undergraduate laboratory practices for the bacterial growth, the newly synthesized medium was named 'Fruit Waste Agar'. When compared for the growth of the bacterial culture with that commercially available media (Nutrient Agar), it was observed that the test organism (*Lactobacillus bulgaricus*) grew faster and luxuriantly on fruit waste agar than the commercially available (Nutrient Agar) media in the market. In fact, fruit waste agar took only 24 hours of incubation for the growth of bacteria, unlike the nutrient agar (NA) media requires 48 hours. Thus use of this medium can prove to be very cost effective and environmental friendly.

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 grams of Nutrient Agar (NA) powder (HiMedia) costs approx. Rs. 1000/- in India. Thus 1kg of this media costs around Rs. 9 to 10k. The agar-agar powder of 500 grams quantity cost for approximately Rs. 4000/- in India. Considering the cost of agar-agar powder used to formulate the fruit waste agar and all the procedures involved in it, it can be easily estimated that the fruit waste agar is a way cheaper than commercial media.



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