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COMPREHENSIVE REVIEW ON COMMONLY USED CULTURE MEDIUM FOR THE DIAGNOSE OF DIFFERENT SPECIES OF BACTERIA AND FUNGUS

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Abstract *Microbes are the unicellular microscopic organisms. They are found in the form of colonies or as a single cell. Naturally microbes are present everywhere on earth but they can also culture in laboratory. For the growth of microbes commercially available culture media are very expensive and mostly are not available. So researchers are trying to use low cast bio-products for the preparation of culture media. Main components of media are Carbon, Nitrogen, Sulphur, Phosphorous and many other components like minerals etc. Culture media are in different states i.e. liquid, semi-solid, solid that are used in growth and identification of microbes in the lab. Bacteria can grow in solid or liquid media however fungi can be grown on media that are enriched with carbohydrates and nitrogen components with acidic pH of five to six and at temperature 15-37°C. Mainly there are two culturing media for the growth of fungi, one is natural media that is composed of natural components such as seeds, leaves, wheat germ etc, it's exact composition is unknown but still it is easy to prepare it while other is synthetic media whose composition is known. Both types of media must contain the source of carbohydrates, N and vitamins. Bacteria can grow on different types of media such as Simple media, Complex media, and Synthetic media. In this review we will discuss different media used in the laboratory for growth and diagnosis of Bacteria and fungus. This review will help the researchers in the field of Microbiology.*

Keywords *culture media,identification,Bacteria,carbohydrates*

INTRODUCTION

BACTERIAL AND FUNGAL GROWTH MEDIA

Microbes are very common and ubiquitous creatures and are very crucial for human survival. The basic to microbial study is the preparation of appropriate culture media. Commercially available culture media are very expensive and mostly are not available. Nowadays researchers are trying to use low cast bio-products. Main components of media are Carbon, Nitrogen, Sulphur, Phosphorous and many other components like minerals etc(Andualem and Gessesse, 2013; Deivanayaki and Antony, 2012; Harvey, 2007; Kasmi *et al*., 2018). Nitrogen is most important and worthy component of bacteria culture media. Main sources of nitrogen are plants, dairy proteins (Annan-Prah et al., 2010; Ummadi and Curic-Bawden, 2008; Muzammal *etal*.,2019).

About 10 of the main components are required for the growth of microorganism i.e. Carbon, Oxygen, Sulphur, Nitrogen, potassium, Hydrogen, Calcium, Magnesium, Phosphorus and Iron. In synthesis of proteins, lipids, carbohydrates and nucleic acid, six elements are involved Carbon, Oxygen, Hydrogen, Nitrogen, Sulphur, Phosphorus and other 4 components involve in number of cell activities (Potassium, Calcium, Magnesium, and Iron). For the activity of cofactors and enzymes many microelements are also required by microbes (Magnesium, Zinc, Cobalt, Nickeletc.). Many growth factors are also required by microbes that are organic in nature.

Culture media are in different states i.e. liquid, semi-solid, solid that are used in growth and identification of microbes in the lab (Seeley and VanDemark, 1962). Many of the microbes doesn't prepare all the amino acids, vitamins and essential many more components. Generally,

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all the media components are in simpler form that microbes absorb easily. Many complex components taken from natural bio products that are used for microbes growth (Kästner*et al.,* 1994).

To form a desirable biomass of microbes on a culture media, recognition of metabolism of microbes are required so that media would maintain the metabolic activity of the microbes (Ummadi and Curic-Bawden, 2008).

BACTERIAL AND DISCLOSURE OF ITS GROWTH MEDIA

Bacteria (sing; bacterium) are kind of Biological cell. Generally, bacteria are several micrometers in length and found in different shapes such as spherical, rod and helical. Bacteria were the first life that was found on earth. Typically they live in aqua media, soil, etc(Fredrickson *et al.,* 2004). The proportion of bacteria is different in different places like 1 gram of soil contains 40 million bacterium and 1 milliliter of fresh water contain one million. However almost 5×10^{30} bacteria are found on earth(Whitman *et al.,* 1998). Huge amount of bacteria are also present in gut as well as on skin of humans and animals (Sears, 2005). Bacteria are prokaryotes they lack membrane bounded organelles and nucleus(Woese*et al.,* 1990).

Bacteria have role in fixation of nitrogen(Tibbett and Carter 2008), at industrial level bacteria are used for sewage treatment, manufacturing of curd and cheese through fermentation process(Deplanche*et al.,* 2010). In field biotechnology bacteria are used for the production of antibiotics and other chemicals(Ishige*et al.,* 2005).

Bacteria can grow at laboratory level(Dudek*et al.,* 2007). Mostly solid or liquid media are used to grow bacteria in laboratory (Thomson and Bertram 2001). In laboratory techniques, high amount of nutrients are used to increase the production of bacteria (Paerl*et al.,* 2001). Bacteriacan grow into the simple, synthetic or complex media.

COMPOSITION OF GROWTH MEDIA

Bacteria are the chemoorganoheterotrophs. To grow bacteria at laboratory, it is essential to deliver all the environmental and nutritional requirements that are found in their natural habitat. So, artificial growing media should deliver all the nutritional components that bacteria gain through natural environment for their growth. Mostly growth media composed of H_2O, source of nitrogen, C and energy and growth factors and trace elements. The pH of the medium is also set accordingly. Certain components of growth media are H_2O, agar, peptone and extract of meat or yeast(Paul *et al.,*1980). Types of bacterial growth media on the basis physical state.

SOLID MEDIA

Solid media contain solidifying agent such as agar at proportion of 0.5 to 2%. Agar is mostly use for the purpose of solidification. It is unbranched polysaccharide and attained through plasma membrane of various species of red algae. The melting point of agar is 95°C while solidifies at 42°C in the form of gel. Agar is might be source of Ca and organic ions(Acharya*et al.,*2013).

SEMI SOLID MEDIA

Semi solid media prepared through reducing the amount of agar from 0.5%. It is soft in nature and mostly used to monitor the movement of bacteria as well as to separate the motile and immotile bacteria(Acharya*et al.,*2013).

LIQUID MEDIA

Liquid media can b prepare in test tubes, glass bottles or conical flask. This media lacks the solidifying agents, such as agar or gelatin however it contains specific proportion of nutrients. Term “broth” is use for liquid media i.e. nutrient broth. Liquid media is mostly used to reproduce large number of bacteria. Properties as well as differentiation between different types of bacteria can’t be determined in liquid media(Paul *et al.,*1980).

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TYPES OF GROWTH MEDIA ON THE BASIS OF INGREDIENTS

Media can be categorized as simple, complex and synthetic (defined) media. Maximum ingredients are same in various media, however some bacteria required additional nutrients. Non fastidious bacteria can grow in minimal requirements however those bacteria that need extra nutrients are known as fastidious(Paul*et al.,*1980).

1. *SIMPLE MEDIA*

Support the growth of non-fastidious bacteriacontains.e.g. peptone, water, nutrient agar. Nutrient broth composed of peptone, meat extract and sodium chloride(Ohta*et al.,*1980). Nutrient agar prepared through by adding 2% agar in nutrient broth

2. *COMPLEX MEDIA*

Rich in nutrient. They contain water dissolving nutrients such as peptone, tryptone. Glucose is used as a source of carbon and energy. However, the actual composition is unknown. e.g. Blood agar

3. *SYNTHETIC MEDIA*

Also known as "define media". In this type of media all components are known while lack tissue of plants and animals and yeast(Magotaet al.,2001) e.g. Davis and Mingioli medium

GENERALLY USED LABORATORY MEDIA

These are divided into six different types:

- ***Basal media***

Basal media is actually a type of simple media that allow the growth of bacteria (non-fastidious) which don't require enrich media. E.g. Nutrient broth, nutrient agar and peptone water.
Staphylococcus and Enterobacteriaceae can grow in basal mediaas in figure 1.

Figure 1: Nutrient agar (Acharya et al., 2013)

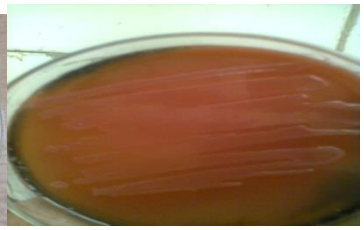

Figure 2: Blood agar

Figure 3: Selective media

- ***Enriched media***

By adding extra nutrients such as blood, serum etc into the basal media made them enriched media e.g. Blood agar, chocolate agar.
In blood agar media Streptococci can grow as in figure 2.

- ***Selective media***

This type of media allows the growth of specific bacteria while inhibit the growth of others. Selective media allow the growth of gram-negative bacteria for example Enterobacteriaceae(Leifert*et al.,*1992)Antibiotic are use as inhibitory agent in this media. Examples of this media includesMacConkey agar, Lowenstein-Jensen media, tellurite media as in figure 3 (Bengval, 1995).

INDICATOR OR DIFFERENTIAL MEDIA

Differential media is used to differentiate the microorganism from one another on same media. Dyes or metabolic substrates etc. are added in the media that are utilized by bacteria and give different color appearance to their colonies. e.g. MacConkey's agar, cystine–lactose–electrolyte-deficient agar, Thiosulfate-citrate-bile salts-sucroseagar, Xylose Lysine Deoxycholate agar etc. as in figure 4(Washington, 1996).

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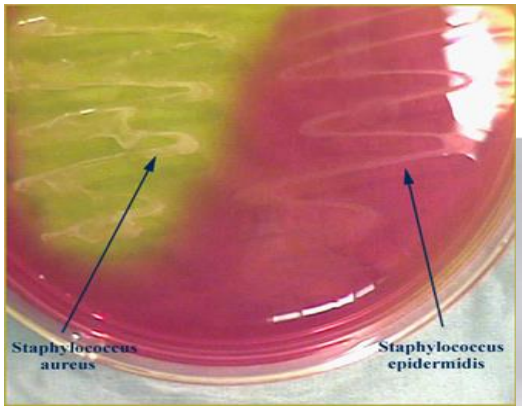

Figure 4: Differential media (AcharyaFigure 5: Cary-Blair medium (Brown et al.,2011)et al.,2013)

TRANSPORT MEDIA

This type of media is use to maintain the specimen when it don't cultured immediately after collection(Acharya*et al.,*2013).E.g. Examples: Cary-Blair medium, Amies medium, Stuart medium as in figure 5.

ANAEROBIC MEDIA

This type of media is used for the growth of anaerobic bacteria because they required less oxygen, reduced oxidation reduction potential and additionalnutrients. E.g. Thiglycollate medium as in figure 6(Acharya*et al.,*2013).

F*igure 6: Anaerobic media (Acharya et al.,2013)*

BASIC INGREDIENTS OF THE CULTURE MEDIA

Culture media prepared through combination of different components that perform various functions.

1. *Nutrients: Amino acids: Proteins*

Naegeli was first who describe that microorganism need proteins which he named peptone for their growth. Later studies reveal that group of bacteria called as chemo-organotrophs need amino nitrogen compounds in their culture media as essential growth factor. For preparation of growth media the nutrients chose carefully in order to complete all the needs of microorganism in media (Debergh*et al.,*1983).

2. *Energy: $(CH_2O)_x$*

Generally glucose is use as energy in media to raise the growth rate of microorganism. Carbohydrates are added in the media by the ration of 7-11g/liter (Debergh*et al.,*1983).

3. *Essential metals and minerals*

There are various essential components of growth media that are catogerozied on semi-quantitative basis(Debergh*et al.,*1983).

4. *Macro components*

Sodium, Potassium, Chloride, Sulphur, Calcium, Magnesium, Phosphorus, Iron

5. *Micro components*

Zinc, Manganese, Strontium, Bromine, Copper, Boron, Cobalt, Molybdenum, Vanadium etc (Debergh*et al.,*1983).

6. *Buffer*

It is necessary to maintain the pH of the culture media to maintain the growth of microorganism. Different buffering agents such as Phosphates, acetates, citrates, zwitterion compounds and specific amino-acids are used in culture media (Debergh*et al.,*1983).

7. *Indicator Substances*

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Adding colored indicators in media is a useful method to check the fermentation of particular carbohydrates in culture media. Phenol red, bromo-cresol purple, fuchsin, etc. are used as indicator. These are toxic in nature so it is important to use fewer amounts of pre-screened batches (Debergh*et al.,*1983).

8. *Selective Agents*

Different chemicals are used to make media selective for the growth of specific microorganism. Specific amount of selective agents are used to inhibit the growth of undesired microorganism from polymicrbial sample. Bile salts, dye-stuffs, selenite, tetrathionate, tellurite and azide are the chemicals that are mostly used as selective agents (Debergh*et al.,*1983).

9. *Gelling Agents*

In some particular media gelatin is used as gelling agent till day. Sometime carrageenans, alginates, silica gel and polyacrylamides are also used as gelling agents but agar is all time super to prepare jelly structure in media. Agar is obtained from agarophyte sea-weeds and commercially available in powder form (Debergh*et al.,*1983).

FUNGAL GROWTH MEDIA

Fungi can be grown on media that are enriched with carbohydrates and nitrogen components with acidic pH of five to six and at temperature 15-37℃. Mainly there are two cultural growth media for fungal growth. One is natural media that is mainly consist of natural components (seeds, leaves, wheat germ, corn meal, stems, oatmeal etc. Despite of unknown composition of natural media still it is easy to prepare. Second the synthetic media which have known composition. These media have known composition of carbohydrates, N and vitamin sources.
For cultivation of fungus, number of media should be used:

BRAIN-HEART INFUSION AGAR

It is non-specific media that is used for all type of relevant fungi growth. Mainly used for the recovery of saprophytic fungus and dimorphic fungi and also for fastidious microbes.

Composition

Brain heart infusion media is made up of pig and beef heart and also calf brain can be used 17.5g, gelatin enzymatic digest 10g, disodium phosphate 2.5g, dextrose 2g, NaCl 5g and agar (Atlas and Parks, 1993; Broth; Ronald, 2004) as in figure 7.

Figure 7.Brain heart infusion agar mediaFigure 8.Czapek's agar (Ho et al.,1986)

This media use for the growth of pneumococci, meningococcal, streptococci etc.(Cheeseman and Hood, 2017).

CZAPEK'S AGAR

It is differential media used for the recovery of *Aspergillus*speicies, soil bacteria, and saprophytic fungi as in figure 8.

Composition

Sucrose 30g, sodium nitrate 2g, dipotassium phosphate 1g, magnesium sulphate 0.5g, ferrous

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sulphate 0.01g, agar 15g (Booth, 1971).

INHIBITORY MOLD AGAR

This media is used for the cultivation and recovery of dimorphic fungi while saprophytic and dermatophytes could not be recovered from this media (Scognamiglio *et al.,* 2010).

Composition

Tryptone 3g, Beef extract 2g, yeast extract 5g, dextrose 5g, starch 2g, dextrin 1g, chloramphenicol 0.125g, salt A 10ml, salt C 20ml, agar 17g, distilled water 970ml (Fischer and Kane, 1968) as in figure 9.

Figure 9. Growth of candida albican on inhibitory mold agar (Maurya et al., 2017).

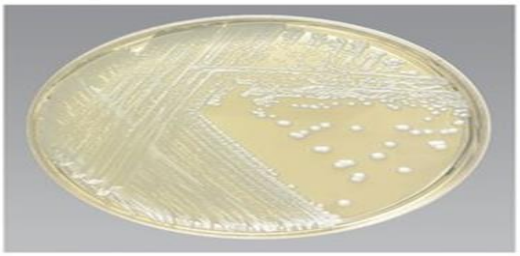

Figure 10: Mycosel agar media (Rhodes et al.,1968)

MYCOSEL/MYCIBIOTIC AGAR

It is actually sabouraud's dextrose agar with addition of cycloheximide and chloramphenicol and used for dermatophytes. While Niger seed agar used for the identification of Cryptococcus neoformans.

Composition

Mycosel agar (dehydrated) 36g, water (distilled) 1000ml as in figure 10.

PER LITRE FORMULA

Papaic digest of soybean meal 10g, dextrose 10g, cycloheximide 0.4g, chloramphenicol 0.05g and agar 15g (Fischer and Kane, 1968).

POTATO DEXTROSE AGAR

It is basically enriched media that is used for number of fungi.

Composition

Potato 200g, Water one litre, Dextrose 15g, Agar 20g (Basu et al., 2015; Booth, 1971; Rinaldi, 1982).

Use for the growth of *Aspergillus Niger, Saccharomyces Cerevisiae and Candida Albicans*as in figure 11.

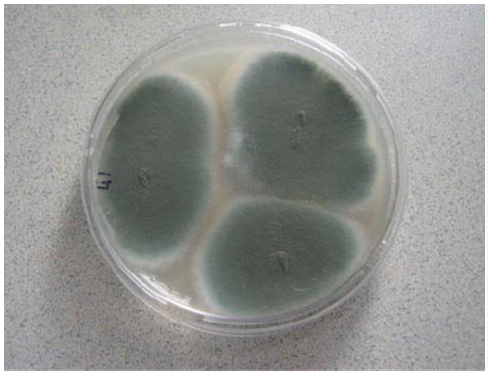

Figure 11.Growth of Penicillium roqueforti on potato dextrose agar(Çon et al., 1996)

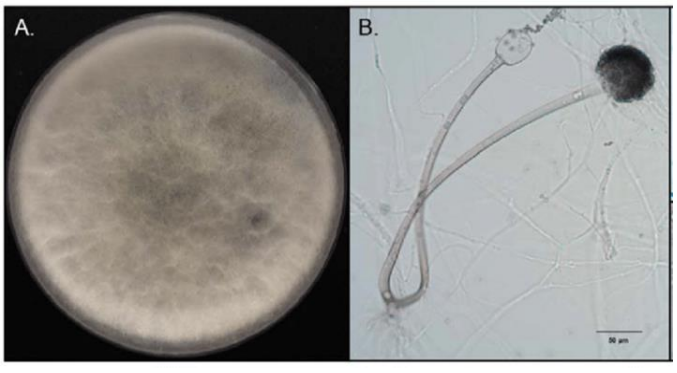

Figure 12. Potato flake agar (Tsyrkunou et al., 2014)

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POTATO FLAKE AGAR

This media is used for the slow growing fungi and for the recovery of spore producing fungi and for dimorphic fungi as in figure 12(Harrigan and McCance, 2014).

Composition

Potato flakes 20g, agar 15g, dextrose 10g, water 100ml

This media is used for the cultivation of candida *Albicans, PencilliumRoquefortii, AspergillusFlavus*etc.

STARCH AGAR

It is a differential media use to check the ability of organism to produce exoenzyme a-amylase etc.(Lal and Cheeptham, 2012).

Composition

Starch (soluble) 40g, yeast extract (marmite) 5g, agar 20g, water one litre (Booth, 1971)

Example it is use for the identification of aerobic actinomycetes as in figure 13.

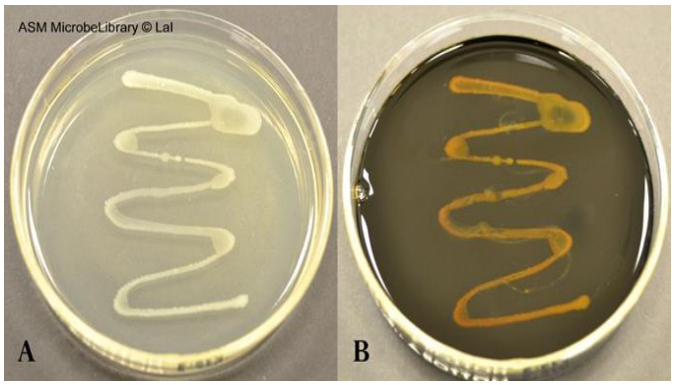

Figure 13. Growth of E. coli on starch agar a. before addition of iodine b. after addition of iodine (Lal and Cheeptham, 2012)

CONCLUSION

In present review, the limited knowledge provides basic about the growth of microorganisms on different types of the growth media. To culture different strains of microbes in laboratory, numbers of media are nowadays in practice. Hundreds of strains are still uncultivatable. To culture those strains unseen factors should be adopted to research. Some of the control factors are missing from the media that largely effect the cultivation of microbe'si.e.Ph. control, temperature control, osmoregulation etc. Alternative agents can be used to cultivate those microbes that are not grown on these media.

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