Culture media for fungi pdf

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Microbial media has undergone several changes since its inception but some key challenges remain. In recent years, there has been exploration of several alternative nutrient sources, both to cater to the specificity in requirement of growth of “fussy microorganisms” and also to reduce costs for large-scale fermentation that is required for biotechnology. Our mini-review explores these developments and also points at lacunas in the present areas of exploration, such as a lack of concerted effort in pH and osmolarity regulation. We hope that our commentary provides direction for future research in microbial media.Microorganisms are almost omnipresent, very diverse and indispensable to human survival. Preparation of suitable culture media is one of the prerequisites to study them. Different microorganisms thrive at different environments and have variety of growth requirements; like nutrients, pH, osmotic conditions and temperature. Due to the lack of sufficient variability of media composition, replication of the exact environmental conditions in the laboratory, nearly 99% of all microorganisms are still unculterable, for example Trephonema pallidum, Candidatus liberibacter, Tropheryma whippelii, Bartonella henselae [1]. Given the current limitations of microbial growth in lab, formulation of newer media should be a much needed thrust area of modern biology.Microbial culture media can be of different type, depending on the nutritional growth requirements of the microorganisms. Microorganisms require about 10 macroelements namely (C, O, H, N, S, P, K, Ca, Mg and Fe). The first six components are used in the synthesis of Carbohydrates, Lipids, Proteins and Nucleic acids and the remaining four exist in the cell as cations and play a variety of roles. In addition to macroelements, all microorganisms require several microelements like (Mn, Zn, Co, Mo, Ni and Cu). These are generally part of enzymes and cofactors. Microorganisms also require growth factors, which are organic compounds.Bacterial media can be formed into simple, synthetic or complex media, where they vary in nutritional make-up. Simple media facilitates the growth of non-fastidious bacteria and the exact chemical compositions of simple media are known. Synthetic media is composed of minimal ingredients needed for the growth of the microorganisms, for example Davis and Mingioli Medium. However in complex media, the exact chemical composition is not known, for example in Tryptic Soy Broth. Bacterial media can be of different consistency, solid nutrient agar medium, Stuart′s semi solid media and nutrient broth liquid media.A pure culture is often paramount for conducting research on particular bacteria and for that, selection of the medium is pivotal to isolate and grow them. Therefore various selective and differential media have been formulated, which have various components that differentiate one group of organism from another, for example, MacConkey Agar and Blood Agar. Different alternative media have been prepared by supplementing with specific components to help in further selective culturing of bacteria, for example, Dorset medium and Petergnani medium used for cultivating mycobacteria. Dorset medium consists of egg white and egg yolk and a solution of sodium chloride [2]. However, besides mycobacteria, egg-based media have also been found to support growth of certain other pathogenic bacteria like Streptococcus pneumonia, Neisseria meningitides and Haemophilus influenza (type b) [3]. Starch and protein substitutes like cow pea, green gram and black gram have also been used to reduce the cost of microbial media.Media containing high carbohydrate source, nitrogen source are required for the growth of fungi at pH range of 5 to 6, and a temperature range from 15 to 37˚C. There are two general types of fungal culture media: natural and synthetic. Natural media are composed of natural substrates, such as herbaceous or woody stems, seeds, leaves, corn meal, wheat germ, and oatmeal etc. Natural media are usually easy to prepare but they have the disadvantage of their unknown composition. Some examples include corn meal agar, potato dextrose agar, V-8 juice agar, and dung agar. Synthetic media, on the other hand, contain ingredients of known composition. These types of media can be duplicated with precision each time they are made and contain defined amounts of carbohydrates, nitrogen, and vitamin sources. Czapek-Dox medium, glucose-asparagine and Neurosporacrassa minimal medium fall in this category. General purpose media, which are commonly used for fungal culture, are Sabouraud dextrose agar (SDA) which is nutritionally poor with acidic pH (5.6). Selective media, like Inhibitory mould agar and Dermatophyte test media are important in the isolation of fungal pathogens such as Cryptococcus neoformans and dermatophytes [4]. Agar supplemented by rice, casein, and other nutrients like Cornmeal agar with Tween 80 have been used to differentiate Candida species and Trichophytan species. Potato dextrose agar used to enhance conidia and pigment development of Trichophytan rubrum. Most lab used media are not cost effective at a large-scale, so industrialists have started to use several common cheap sources of carbon.Studying the mechanism behind the robustness of extremophiles has implications for biotechnology, evolution and most interestingly in search for life in extreme conditions. However, conventional culture techniques often fail to support the growth of extremophiles under high temperature and pressure conditions, low or high pH values etc. Culturing of thermophiles cannot be done on cellulose and agar plates containing Luria-Bertani broth or nutrient broth, as these are unstable above 70˚C for extended periods of time [5].Solid culture technique using porous solid plate made of nanofibrous cellulose has been developed recently which can retain its integrity up to 260˚C at 25 MPa [5]. This finding provided a versatile platform to support the growth of a wide number of extremophiles including acidophiles, alkaliphiles, thermophiles, acidothermophiles and alkalithermophiles under extreme physiochemical conditions in laboratory.In the earlier days, isolation of bacteria for preparation of pure culture was very difficult in liquid media. Often any attempt to prepare pure culture using liquid media would result in contamination because of the slow and tedious steps. Thus came the need of a solid media, which was achieved by adding a gelling agent to the liquid broth. The first gelling agent used was gelatin by Robert Koch in 1881 to prepare solid medium.But as it melted at a temperature around 35˚C and was digested by the bacteria, he faced problems with its use. These problems led to the discovery of an alternative gelling agent agar. Angelina Fanny Eilshemus, wife of Walther Hesse, an associate of Koch, first proposed the use of agar as a substitute to prepare solid culture medium. Gradually various advantages of agar made it very popular amongst the scientists, as it is stable at wide range of temperature (solidified at 32˚C to 42˚C, melted at 85˚C) and thus is suitable for the growth of mesophilic organisms. Additionally agar does not have any toxic effect on bacteria, has good diffusion characteristics and is not digested by most bacteria. Agar is also found to have good clarity and is metabolically inert. But on the flip side, agar is not suitable for culturing thermophilic microbes and inhibits PCR in a concentration dependent manner [6]. Besides, high cost of agar has made research work very expensive and due to its high usage in laboratories, the natural resources of agar (i.e. Gelidium sp., Gracillaria sp. and Pterocladia sp.) are being over-exploited and have made scientific community to look for some other alternative sources of gelling agents.pH of the culture media effects the growth of microbes. Any organism has an optimal pH at which it grows the best. Alteration of this pH value leads to undesirable growth. For most bacteria there is an orderly increase in growth rate while approaching the optimum “pH” value and a corresponding orderly decrease in growth rate while it starts to vary from the optimal point. Each species of microbe is divided into three types on the basis of its own characteristic range of pH values in which it grows and reproduces best. Although we know that the general pH range for most bacteria is 5 to 9 (optimum 7), they can be acidophilic (pH range from 0 to 5.5), neutrophilic (pH range from 5.5 to 8.0) and alkalophilic (pH range from 8.5 to 11.5) [7].Just like other organisms, microorganisms also need a physiological pH inside their cells. The ability to survive in extreme pH either high or low, depends on their ability to neutralize the environmental difference with the physiological pH [8]. Microorganisms like Helicobacter pylori mostly are found in stomach in very acidic conditions, so to maintain their environmental differences they produce urease, an enzyme that degrades urea and decreases the acidity [9]. There are other bacteria that are specialized to live in alkaline pH, for instance near black smokers, geological fountains of minerals that shoot highly alkaline minerals into the ocean [10].The change in the acidic or alkaline conditions of the medium is indicated by a color change using pH indicators. They are used in culture media to differentiate between the different groups of microorganisms based on their acid or alkali producing properties. For example, Eosin and Methylene blue, Bromothymol blue, Acidfuchsin, Phenol red etc [11].Osmolarity is an important parameter for the formulation of any culture media as it helps the media resemble the natural environment of the cells for their proper growth. Certain organic solutes known as osmoprotectants are used to make the cells resistant towards a wide range of osmotic tension. So far, in microbial growth osmolarity has not been systematically explored as a parameter to culture unculturable and difficult to culture microorganisms.Several metabolically active microorganisms that can be seen under microscope, cannot be cultured in the laboratory conditions. To grow these unculturable microorganisms novel techniques have been implemented in recent times. Some bacteria exhibit co-culture dependence. They only grow in the presence of helper microorganisms, and present a problem of a need of mixed culture.The current knowledge base of microbial growth media is a limiting factor in culturing various microorganisms. Broadening the knowledge base will require extensive research in the field and help achieve affordable culturing of the unculturable microorganisms. Better control of pH, use of better pH probes and control over osmolarity is largely missing from current microbial media. We expect to see a systematic research in this direction to culture presently unculturable microorganisms. A look into animal cell culture, which has been focus of more recent research, can guide further development of microbial media. It is possible that alternative gelling agents can support the growth of microorganisms that do not grow on agar. A systematic evaluation of gelling agents is much needed. We expect microbiology to have another “golden age” with resurgence in interest in growth media.Citation:Basu et al, Bioinformation 11(4): 182-184 (2015)1. Bhattacharya S, et al. Indian J Med Microbiol. 2002;20:174. [PubMed] [Google Scholar]2. Wasas AD, et al. J Clin Microbiol. 1999;37:2045. [PMC free article] [PubMed] [Google Scholar]3. Wasas AD, et al. J Clin Microbiol. 1998;36:1139. [PMC free article] [PubMed] [Google Scholar]4. Collins MM, et al. 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