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A Review: Formulation of Alternative Culture Media

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ABSTRACT: Culture media is the inevitable part of study of microorganisms and plays a significant role for flourishing microbial growth and metabolism. It consists of variety of macro as well as microelements. It can be raw materials or the synthetic media. However, traditional culture media components, especially synthetic media components are costly. As a result, numerous researches have been attempted to create and assess the effectiveness of alternative, affordable culture media, often employing basic materials that are natural and easily accessible. The current study reviews at several plant-based culture media formulations for microorganism growth and the generation of industrially relevant microbial chemicals. Most formulations which have been studied includes horticulture ingredients, vegetable substrates including soy, certain beans, maize and rice. In terms of microbial growth efficiency and manufacturing cost, alternative culture media frequently produce results that are satisfactory compared to standard media.

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INTRODUCTION

Food waste nowadays is generated in a bulk amount which is not at all sustainable to the economy of the country, hence methods of reusing it have been continuously developed and improved (Ramurez *et* al., 2020). Interestingly, the scientific community has put great efforts in the development of methodologies for the use of various types of food waste, whether of animal or vegetable origin, in order to obtain new products or compounds of interest or to optimise processes (Navarro-Peraza *et* al., 2021, Leria *et* al., 2019).

The nutrients required for microbial development are provided by culture media. For making these media, which can be used for microbial growth or for other reasons, such as selective, differential media and media used to promote the production of microbial compounds, a variety of raw materials are available (Rouf *et* al., 2017).

As a source of fibres, polyphenols, and bioactive compounds in general (Costa *et* al., 2019), organisation and management of fruit and vegetable residues for human and animal consumption (Ramurez *et* al., 2020), and use in the creation of alternative culture media, agro-industrial waste is extremely diverse and has the potential to be applied in many different fields.

In institutions with insufficient financial resources, practical microbiology classes and scientific researches are hampered in underdeveloped nations by the high cost of culture medium (Uthayasooriyan *et* al., 2016, jadhav *et* al., 2018, cruz *et* al., 2020). Similarly, some researches have indicated that vegetable-based formulations may be useful as substitute (non-commercial) culture media since these substrates offer a variety of nutrients, including proteins, necessary for the growth of microorganisms (Santos *et* al.,2021). These techniques, which are still in development, attempt to increase sustainability by using waste materials or food plant components that aren't frequently utilised to make food for humans, including vegetable peels and stems.

In educational and research institutions with financial constraints for the procurement of conventional cultural media, the goal is to offer a scientific basis for, and support the production and use of, these culture media. Present review sheds light on novel researches on alternate culture media made using plant-based ingredients.

ALTERNATIVE CULTURE MEDIUMS FOR MICROBIAL GROWTH

Culture media is a gel or liquid that contains nutrients and is used to grow bacteria or microorganisms. They are also termed growth media. Different cell types are grown in various types of medium. Nutrient broths and agar plates are the most typical growth media for microorganisms. The right temperature and sufficient nutrients are essential for the growth of microorganisms.Regarding the latter, when used in culture media, the elements of various vegetables can offer the essential microbial nutrition. Alternative culture media could be categorised based on their objectives by evaluating scientific publications: microbial growth media and media for microbial compound synthesis.

Microorganisms require a number of favourable conditions in order to flourish, including the right temperature and sufficient nutrition. Bacteria just require water, a carbon source, a nitrogen source, and some mineral salts in order to grow (Kumar *et* al., 2012).

Water: Water is essential for the solubilization, transportation, and maintenance of hydrolysis reactions of nutrients. For some bacteria to flourish, free water is necessary. This water may be lost if evaporation takes place while the agar is being incubated, which would reduce the size of the colony and impede bacterial development ((Kumar *et* al., 2012, Power *et* al., 2009).

Carbon source: In bacteria, carbon is the most prevalent element. Producing food molecules including lipids, carbohydrates, proteins, and nucleic acids is crucial for bacteria. In addition to using organic sources like sugars and alcohols, bacteria can also utilise inorganic carbon sources like carbon dioxide ((Kumar *et* al., 2012, Atlas *et* al., 2010).

Nitrogen source: Regarding nitrogen sources, there are several of them and you can find them in a lot of the compounds that make up a growth medium. It can be present in both an organic form, which corresponds to protein hydrolysates, especially when the hydrolysate is proteose-peptone or tryptone (Atlas *et* al., 2010), and an inorganic form, which is nitrates (Latge *et* al., 1975). Protein synthesis is made possible by nitrogen in bacteria. Finally, phosphate, sulphur, magnesium, or calcium (Power *et* al., 2009) are frequently found among the common mineral salts.

Energy source: There are two types of bacteria: chemotrophic bacteria, which use the energy of oxidation of mineral or organic compounds as energy sources (Pasteur et al., 1922 Cook *et* al., 2018), and phototrophic bacteria, like *Thiocapsa roseopersicina* (Yurkov *et* al., 1998), which convert light into an electrochemical gradient of protons (van der Horst *et* al., 2007).

In investigations on microbiology, microorganisms are cultivated by adding proper culture media and a favourable atmosphere in a lab setting (Jadhav *et* al., 2018). The majority of the time, readily accessible commercial media like Cetrimide agar, Nutrient agar, and MacConkey agar are utilised, but these are typically thought of as expensive media (Jadhav *et* al., 2018). There have been reports of the growth and isolation of organisms utilising various substrates and mediums (Basu *et* al., 2015). Some foods, including cabbage, carrots, tomatoes, pumpkin, and gooseberries, have been used in place of nutrient agar to cultivate both bacteria and fungi (Deivanayaki *et* al., 2012). To reduce the cost of microbiological medium, some reports have substituted green gramme, cowpea, and black gramme for starch and protein (Wasas *et* al., 1999). Fruit and vegetable bio-waste contains simple and complex sugars that are broken down by microbes. These sugars have drawn interest because they can be used to produce biogas, ethanol, and animal feed (Tijani *et* al., 2012, jamal *et* al., 2013).

In order to create low-cost growing media for microorganisms, various types of agricultural waste are being utilised (Kahraman *et* al., 2002, Milala *et* al., 2005). Additionally, agar of Dragon Fruit Peel (DFP) was employed as a medium for microbial growth (Putri *et* al., 2017). High concentrations of carbohydrates found in grapefruit, banana, and melon peels serve as an excellent substrate for the synthesis of amylase (Siddiqui *et* al., 2014). A cheap and efficient substrate for the growth of fungus was discovered to be banana peel (Kindo *et* al., 2016). The extract from the watermelon peel is abundant in macronutrients such lipids, reducing sugars, and total proteins (Hasanin *et* al., 2020). According to the study, watermelon peel waste (WPW) grew *Aspergillus niger*, *Fusarium oxysporum*, *Lichtheimia corymbifera*, *Penicillium expansium*, and *Rhizopus oryzae*, among other organisms, the best. The study also showed that formulated watermelon peel waste dextrose agar (WPWDA) medium was discovered as an alternative way for some commonly used media such as Czapek's Dox agar (CzDA) and Potato dextrose agar (PDA); in addition, this medium was extremely affordable and environmentally friendly. When using oleaginous yeasts to produce biodiesel from orange peel waste (OPW), it was discovered that the strains of *Rhodosporidium toruloides* NRRL 1091 and *Cryptococcus laurentii* UCD 68-201 produced 31, 9% and 36, 9% of the biodiesel, respectively (Carota *et* al., 2020). Cellulase enzyme was produced by *Trichoderma reesei* using pea peel, sponge gourd peel, lychee peel waste as a growth medium at 30 C (Verma *et* al., 2011, Verma *et* al., 2018). Orange peel, Potato peel, drumstick peel powder used for the growth of *Trichoderma* spp. and *Aspergillus* spp (Kadam *et* al., 2017).

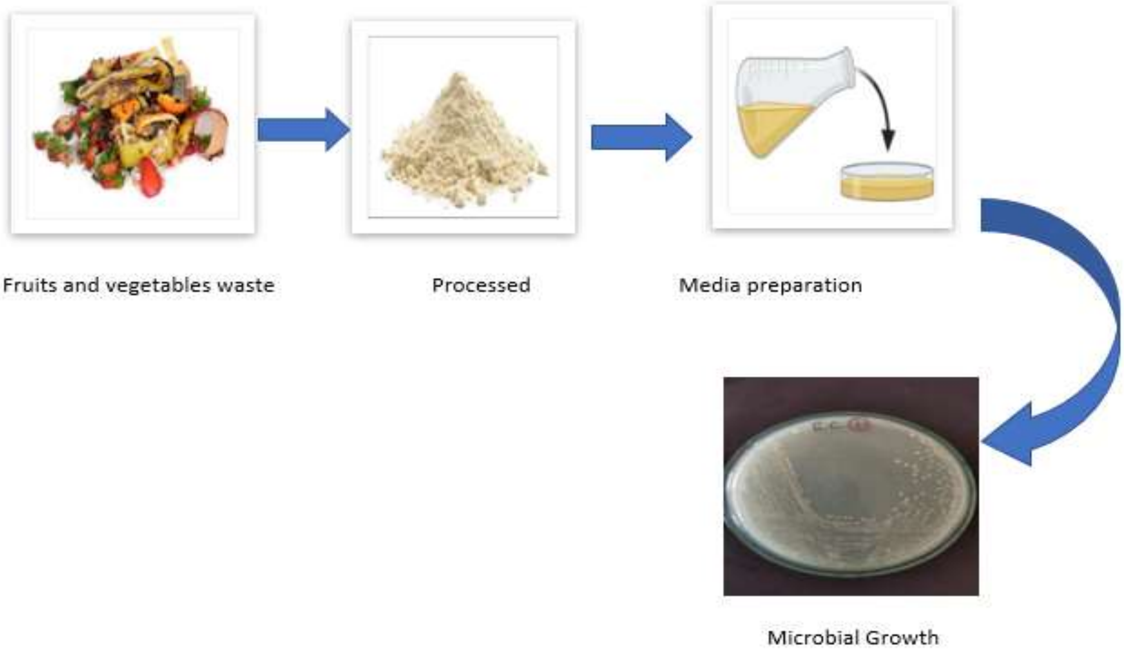

Figure: 1 Alternative media formulation

In addition to having a high nutritional value, soy is a significant export from Brazil that contributes significantly to the country's economy (Cattelan *et* al., 2018). Regarding its use in culture medium, soy is one of the most adaptable foods. Soy is a major source of nutrients in many commercial media, hence its application in alternative culture media has been thoroughly researched.

In a study that was published by our group, textured soy protein (PST), which is widely available, was utilised in concentrations between 0.5 to 10 percent in the creation of alternative culture media. The media containing various concentrations of PST were infected with 38 bacteria, including significant pathogens and bacteria associated with food spoiling, such as *Bacillus cereus, Micrococcus luteus, Escherichia coli, Hafnia alvei,* and *Serratia marcescens*. All of the examined bacterial species were able to grow at a concentration of 7.5%. In addition to being successful for microbial growth, the medium's production cost was 86% lower than that of tryptone soy broth and 68% lower than that of tryptone soy agar. This research established the viability of a simple-to-make, inexpensive, and highly effective culture medium (Cruz *et* al., 2020).

A culture medium based on soy flour was produced in a study conducted in Sri Lanka to assess the development of yeast strains (*Saccharomyces* sp. and *Schizosaccharomyces* sp. According to the study, the alternative soy flour medium was more effective than the standard culture media at growing yeast. Another benefit is the simplicity of obtaining soy flour through soy milling, which is roughly 50 times less expensive than the commercial medium including peptone and yeast extract (Santos *et* al., 2021).

In a Sri Lankan study, natural and processed soy flour, rice, chickpeas and maize were also employed separately as the foundation for alternative media. The researchers studied various microorganisms, comparing their development in designed and commercial media such potato dextrose agar (PDA) for fungi and nutritional agar for bacteria. In order to create alternative media for bacteriological and mycological studies, it is recommended to use inexpensive and readily available raw materials such rice, chickpeas, maize and soy (Uthayasooriyan *et* al., 2016). The formulated media allowed the examined microorganisms to proliferate.

The growth of lactic acid bacteria in a culture medium based on soy molasses was assessed by Caldeiro et al. (2015). Defatted soy flour was used to make the molasses, which was then cleaned with an alcoholic hydrocarbon solution. The solvent was then expelled, leaving behind the concentrated solutes that eventually became molasses. The *Bifidobacterium lactis* Bb-12 and *Lactobacillus helveticus* LH-13 strains showed the strongest growth in this medium, displaying counts larger than 108 CFU/mL, according to this Brazilian study, which demonstrated that all of the lactic acid bacteria examined could thrive in the soy molasses medium.

Other vegetables besides soy have been included in culture media formulations. Natural vegetable protein was employed to create alternative media in a study carried out in Iraq using a variety of grains, including rice, lentils, peas, chickpeas, soybeans, cowpeas, and mung beans. As per the study data, all media tested were successful in supporting the growth of the tested microorganisms, which included bacteria and fungi like *Staphylococcus aureus, E. coli, B. cereus, Pseudomonas aeruginosa, Penicillium* sp., and *Aspergillus* sp. that are frequently linked to food spoilage or illnesses.

The findings suggests that these alternative media might readily replace traditional culture media given the positive results (Shareef *et* al., 2019). Using maize extract as an alternate growth medium for *S. aureus* and *E. coli*, Gabunia et al. (2019) examined it in the Philippines. The outcomes showed that the substitute medium delivered the same outcomes as the commercial nutrient agar.

For the generation of biomass from the edible fungus *Pleurotuseryngii*, Andrade (2017) discussed the formulation of alternate culture medium. The media were created through submerged fermentation utilising an infusion of cooked vegetables from the Amazon, such as manioc, purple-skinned sweet potatoes, purple yams, and white yams, at a concentration of 20% (v/v), with the addition of 2% glucose. Efforts made in advance produced successful production of biomass, with a focus on methods based on white yam and purple-skinned sweet potatoes.

Fruit peels and vegetable stems are frequently thrown away or used in compost, but they have a high nutritional value and could significantly lower the cost of making culture media because fruits and vegetables contain a lot of starch and proteins (jadhav *et* al., 2018).

An experiment conducted in India used a culture medium called GCO that contained powdered onion, maize and garlic skins. *Candida albicans, Saccharomyces cerevisiae, Penicillium chrysogenum, Aspergillus niger*, and *Trichoderma viridae* were employed to test the GCO medium's effectiveness. Bacteria such as *Bacillus* sp., *Sarcina* sp., and *P. aeruginosa* were also used. The growth of the microorganisms evaluated on this medium was comparable to that on commercial media, according to the authors, indicating that this culture medium is economically favourable for producing goods from microbial growth (Berde *et* al., 2015).

In one study, the growth of *E. coli, Serratia* sp., and *Pseudomonas* sp. was examined in nine formulations that contained various quantities of drumstick (Moringa) seeds and peel, orange peel, potato peel, cauliflower stem, and fenugreek stem. The outcomes demonstrated that all formulations permitted the growth of the tested microorganisms. In three of the nine formulations, the bacterial growth was also higher than it was in nutritional broth sold in stores (jadhav *et* al., 2018).

Microalgae can be grown on alternative media with the goal of producing biomass for human use and aquaculture. Since microalgae are the foundation of the marine food chain and are a source of nutrition for lobsters, prawns and oysters, the latter has significant economic significance (Aversari *et* al., 2018).

Brazilian researchers assessed the capacity of four microalgae species for growth and biomass production in various alternative media, including vinasse, fruit and vegetable waste (prepared by composting fruit and vegetable waste discarded from distribution markets), and media composed of sugarcane waste products. The outcomes showed that the fruit and vegetable waste-based medium was promising for the development of microalgae since it produced more microalgae per unit area than the control media (Calixto *et* al., 2016).

When evaluating the cultivation of microalgae isolated from the Northeast of Brazil, Medeiros et al. (2020) found comparable results utilising an alternative, inexpensive medium prepared with a bio-composite of leftover fruits and vegetables. They discovered that, in contrast to that in the synthetic culture medium, the development of microalgae in this alternative medium facilitated appropriate microalgal growth and increased antioxidant activity as well as the generation of mono and polyunsaturated fatty acids.

ALTERNATIVE MEDIA FOR OBTAINING MICROBIAL PRODUCTS

Although cellulose is frequently produced by plants, some bacteria, such as those of the genera Gluconacetobacter, Sarcina, and Komagataeibacter, also create this polymer. Despite having useful industrial uses, bacterial cellulose production is quite expensive (Garcia-Sanchez *et* al., 2020, Yang *et* al., 2017). The Costa et al. (2017) study evaluated the effectiveness of alternative media in stimulating the production of cellulose by a strain of *Gluconacetobacter hansenii*. These media were made utilising waste products from the food industry, such as sugarcane molasses and corn steeping liquor. Cane molasses was used as an alternate medium, but the results were subpar. Although the efficiency of the medium including maize steeping fluid was only around 73% that of the commercial medium, it still produced the maximum output of dry and hydrated bacterial cellulose mass.

Mango pulp may be used as an alternate growing medium for *Komagataei bacterxylinus* to produce cellulose, according to a recent study from Mexico. The chemical makeup and thermal degradation of the bacterial cellulose produced in the culture medium based on mango pulp were similar to those of cellulose produced in pure sugars. As a result, the researchers claim that the manufacture of cellulose in this alternate medium is feasible and produces a high yield. Additionally, in terms of sustainability, this approach makes use of an agrifood surplus produced in a number of nations and lowers the cost of producing bacterial cellulose (Garcia-Sanchez *et* al., 2020).

The hydrolysis of triacylglycerol ester bonds is catalysed by the enzymes known as lipases, which releases free fatty acids and glycerol. These microbial enzymes are frequently utilised in the commercial manufacture of food, chemical compounds, and medications. In a study conducted in Portugal, microbial lipase production in alternate medium was disclosed. This study employed alternate culture medium made of varying quantities of cane molasses, millets and Russian water (a byproduct of the production of olive oil), with the goal of producing lipases from Candida rugosa. The creation of lipases in a medium including molasses, millets, and Russian waters in concentrations of 1%, 0.4%, and 0.1%, respectively, was found (Freitas *et* al., 2016). This finding suggests that these products may be utilised to generate enzymes, significantly lowering the cost of culture media.

Combinations of legumes were utilised by Mohammed et al. (2020) to create a media that encouraged Serratia marcescens to produce prodigiosin pigments. White beans, fava beans, mung beans, peas, chickpeas, black beans, and lentil powders were used to make the medium. During a 24-hour incubation period at 25–28 °C, the alternate culture medium's solid and liquid forms encouraged the formation of pigment. The authors analysed the medium of each of the seven legumes separately to confirm pigment synthesis, however they found that prodigiosin production had decreased and the colonies had turned light red. The mixture of legumes used to create the medium turned out to be simple to make and store. Additionally, it was quite successful in promoting S. marcescens pigment synthesis.

APPLICATIONS OF CULTURE MEDIA

Microbial culturing is a fundamental technique employed in various fields of microbiology for a multitude of purposes. One of its primary applications is the identification of the causative agents behind infections, enabling healthcare professionals to diagnose and treat patients effectively. Additionally, culturing allows scientists to study and understand the unique characteristics of different microorganisms, aiding in taxonomy and classification. Isolating pure cultures is crucial for conducting in-depth research and creating stock cultures for future studies. By observing biochemical reactions, researchers gain insights into the metabolic capabilities of microbes.

Furthermore, culturing plays a pivotal role in testing for microbial contamination in various samples, be it in food, water, or clinical specimens. It also serves as a tool to evaluate the effects of antimicrobial agents and preservatives, helping to enhance the safety and shelf life of products. The visual observation of microbial colonies, including their color, shape, and size, is vital in preliminary identification, enabling researchers to differentiate between different types of colonies.

Moreover, microbial culturing is instrumental in producing antigens for laboratory use, supporting the development of diagnostic tests and vaccines. Estimating viable counts allows researchers to quantify the number of viable microorganisms present in a sample, which is essential for quality control and understanding population dynamics. Lastly, testing antibiotic sensitivity aids in determining the most appropriate treatment options for specific infections, contributing to better patient outcomes. In conclusion, microbial culturing is a versatile and indispensable technique that continues to advance our understanding of microorganisms and their interactions with the world around us.

CONCLUSION

The development of efficient and affordable cultural media holds great promise in the realm of scientific investigations. While commercial cultural media are readily available to well-funded institutions, their reliance on food products or components not fit for human consumption can be a limitation. However, the emergence of alternative cultural media that utilize food waste presents an exciting opportunity to broaden access to critical information and promote sustainability. The utilization of food waste in cultural media not only addresses environmental concerns by reducing waste but also offers a cost-effective solution for research and experimentation. By repurposing food waste for this purpose, researchers can make significant strides in resource conservation and contribute to a more sustainable scientific practice. Moreover, the growing body of research supporting the efficacy of alternative cultural media suggests that they can be just as effective, if not more so, than traditional culture media. By embracing alternative cultural media, researchers can explore new possibilities and achieve comparable or even improved results in their experiments. This can lead to novel insights and advancements in various fields of science, including microbiology, biotechnology, and environmental studies. As a result, the exploration of unused food and food waste as cultural media represents a hopeful and promising avenue for scientific progress and sustainable practices. It not only enhances accessibility to scientific tools but also aligns research efforts with a more eco-friendly approach, benefiting both the scientific community and the planet as a whole.

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