VIGNA RADIATA (MUNG BEANS) AS AN ALTERNATIVE CULTURE MEDIUM FOR TRYPTICASE SOY AGAR

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ABSTRACT: High costs of commercial culture media poses challenge in microbiology research, driving a quest for cost-effective culture mediums. The study investigates the potential of Mung beans (Vigna radiata) as a potential alternative culture medium for Trypticase Soy Agar (TSA). This study utilized a quantitative experimental research design with the use of Absolute Growth Index (AGI) Scale and Centers for Disease Control and Prevention (CDC) categorized characteristics of colony morphologies. Mung beans are ground into powder, mixed with 1.5% agar, and processed similarly to TSA. Four-quadrant streaking is performed, followed by incubation at 37°C for 24 hours. Test microorganisms include Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. Colony growth from both the formulated Mung Bean Agar (MBA) and TSA were scored according to AGI and compared with the use of two-way ANOVA. Colony morphology observations reveal that Mung Bean Agar (MBA) produces pinpoint, smooth, grayish, opaque, punctiform colonies with entire margins, whereas TSA yields small/medium, yellow, mucoid, transparent, circular colonies with entire margins. Statistical analysis shows no significant difference in AGI between MBA and TSA. In conclusion, MBA can serve as a cost-effective alternative to TSA for microbiological culture, offering a similar growth performance while being more economically feasible.

KEYWORDS: *Mung Beans, Alternative Culture Medium, Trypticase Soy Agar*

1.0 INTRODUCTION

The ability to cultivate and maintain microorganisms in the laboratory by supplying the right culture medium that provides a favorable environmental condition is the foundation of microbiological studies [1]. As the ever-increasing demands of culture media continue to grow, the dehydrated culture media was reported to hold the largest market share in 2021 globally [2]. The primary factor that drives this demand is the surge in need for innovative vaccines, antibiotics, and oral insulin. According to trade statistics from 2023, 69% of the culture media products in the Philippines are total imports from largest culture media suppliers in China, the United States, and Thailand [3]. The lack of local companies and supply chain constraints made the ready-to- use and quality-assured culture media either prohibitively expensive or completely unavailable in LMICs [4]. Thus, local researchers seek a cost-effective alternative culture medium for commercially produced culture media. [5] Across the countries, the growth in agricultural production has allowed food to become more abundant and cheaper. Fruits and vegetables served as dietary guidance due to their vitamin, mineral concentration, and phytochemical compositions.

Mung bean, scientifically known as Vigna radiata, has been long recognized as an inexpensive and readily available source of balanced nutrients, including protein, minerals, vitamins, and dietary fiber, as per Hou et al. [6]. In the review study of Tang et al. (2014), mung beans contain about 50-60% carbohydrates and 20-24% protein, where the rest of the constituent percentage accounts for minerals, vitamins, and fat. The main storage proteins of mung beans are globulin and albumin which accounts for 60% and 25% of the total protein, respectively [7]. With its excellent source of protein, mung beans have an ideal essential amino acid profile [8]. It is rich in essential amino acids such as leucine, isoleucine, and valine. Mung beans have more excellent carbohydrate content than soybeans which are present in Trypticase Soy Agar [9]. The predominant carbohydrate found is starch. The other

carbohydrates are made up of oligosaccharides such as raffinose, stachyose, and verbascose The development and evaluation of culture media derived from easily accessible raw materials as an alternative to commercial culture media has been the focus of numerous studies over the past few years [9].

The potential of mung beans was investigated by Fadhilah et al. (2022) [10] as an alternative culture medium for bacterial growth using a pure isolate of *Bacillus subtilis* through the streak quadrant method. The findings revealed that the mung bean medium provides a favorable environmental condition comparable to that of Nutrient Agar, which is used as a control medium, enough to support the growth of *B. subtilis* [10]. In addition, a study reported by Berde and Berde [11] revealed that using vegetables to make an alternative culture media can be beneficial given that they consist of several nutrients necessary for the development of microorganisms. Since many of these media involve food products or portions of food products that are not intended for human consumption, institutions can access them with resources sufficient for commercial culture media. By utilizing food waste, formulating alternative culture media supports sustainability in addition to advancing access to knowledge as per Santos et al. [12].

Most cheap protein sources (soy protein, cowpea, lentil, chickpea, mung beans, and split pea), except for rice-based medium, were influential in promoting the growth of the tested microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Penicillium sp*., and *Aspergillus sp*.. Shareef (2019) reported that all tested microorganisms had significantly high growth rates on most protein sources mentioned except rice. *B. cereus* exhibited high growth and large colonies on formulated media. *E. coli* and *P. aeruginosa* showed good growth, while the Pigmentation of *P. aeruginosa* was unaffected. On the other hand, *B. cereus*, *S. aureus*, and *P. aeruginosa* produced beta hemolysis on all formulated agar media. Using protein sources as alternative culture media in laboratories are

feasible and cheaper than commercially manufactured culture media [13]. Arulanantham et al. observed that legume seeds are a rich source of protein for nutritional purposes. Legume seeds (Cowpea, green and black gram, and soya meat) were used in their study as an alternative nutrient source to grow bacteria. Results showed that *Klebsiella sp.* typically grows the least in the protein formulations studied, while *Staphylococcus sp.* grows well in all protein formulations. All the tested bacteria demonstrated more significant growth in nutrient agar across the other media used. In addition, the number of colony growths indicates that Soya meat agar (soya meat + agar) is an effective alternative culture media source to nutritional agar for growing bacteria [14]. *Staphylococcus aureus* and *Escherichia coli* can thrive in corn husk extract, according to a study conducted in the Philippines by Gabunia et al. The results revealed that the corn husk extract was an effective culture medium and produced results similar to nutrient agar sold commercially [15]. The viability of other culture media was evaluated by Uthayasooriyan et al. (2016) using cheap protein sources (rice, chickpea, corn, dhal, thinai, natural soy flour, and processed soy flour) as an alternative medium to Potato Dextrose Agar and Nutrient Agar. *Klebsiella sp*. exhibited more growth for the tested bacteria, while *Bacillus sp.* grew less in all alternative culture media. For tested fungi, *Sclerotium sp.* demonstrated significantly ($p<0.05$) higher growth in rice, while *Penicillium sp.* showed especially ($p<0.05$) low growth in rice and corn. The authors also utilized soy flour as a culture medium, but the results were more satisfying with the fungi than with the bacteria tested [1]. Mohammed et al. [16] utilized mixtures of seven different legumes to formulate an alternative culture media for the growth and stimulation of prodigiosin pigment production of *Serratia marcescens*. To prepare the culture media, white beans, fava beans, mung beans, green peas, chickpeas, black beans, and lentils were powdered and sieved. The color intensity of prodigiosin was used to compare the pigment production. Among the legumes tested, mung beans are one of the legumes that supported the maximum stimulation rate of

pigment production of *S. marcescens*, but the mixture of all legumes shows the highest production. The study proves that legumes are an excellent source of protein and can be used as alternatives for bacterial growth [16]. Gram beans, a member of the legume family, is known to be rich in proteins enough to support the growth of microorganisms in various studies. Black, yellow, green, and horse gram were used as an alternative culture media in the study of Raju [17]. The study did not involve any quantification of the number of colonies present in alternative solid culture and focuses on the comparison of the presence and morphology of *E. coli* and *S. aureus*. Before the microbial inoculation onto the alternative culture media, the researcher prepared a microbial culture of *E. coli* and *S. aureus* from the original stock culture to ensure its purity and freshness. Result shows that there weren't any significant variations in the morphology of the colonies between the alternative gram beans media and NA [17]. Moreover, the use of almond — known to have higher nutritional value compared to other nuts — to replace peptone and meat extract on Nutrient agar was performed by Nurmalasari et al. [18]. The identity of *Escherichia coli* and *Staphylococcus aureus* was confirmed by the researchers through gram staining. Although colony morphology of the alternative media was quite similar to NA, the number of colonies of *E. coli* and *S. aureus* being observed in alternative almond media was less as compared to NA. Subsequently, almond media as being a complex media, the researchers pointed out the several possible causes of the microorganisms' growth. One possible cause includes the boiling process of almonds during media preparation. Boiling the almonds can lead to reduced or loss enzyme activity, solubility changes, and denaturation of proteins causing its decreased level. As a result, decreased nutrient content in alternative almond media leads to impeding growth of bacteria [18]. Among the local plant materials used by Oledibe et al. (2023) [19] is *Glycine max*, or locally known as soybeans. Soybeans are a member of legume family that is present as one of the components in Trypticase Soy Agar. The study focuses on

the evaluation of the *P. expansum* and *A. niger* colony, sporulation, and aerial mycelia growth using the combination of *Zea mays, Dioscorea dumetorum,* and *Glycine max* as alternative culture medium to Sabouraud Dextrose Agar. One of the procedures being carried out in the preparation of alternative culture media is washing thoroughly the *Glycine max* seeds with clean water and dried 3 hours at 60°C in the oven. Dried sample was then blended into powder to enhance solubility and sieved until it reached a flour consistency. The rest of the preparation is just like any commercial culture medium and was autoclaved. Visual evaluation of various media consistency on plates was also done. The alternative culture media showed a strong gelling ability but slowly liquified after a few days. The researchers pointed out that it could be due to a pH decrease while autoclaving that reduces the gel stability. Overall, the colony diameters of test fungi were not significantly different from SDA, remarkable mycelia growth and sporulation were also observed [19].

In this current study, the formulation of alternative culture media out of mung beans will be compared to that of Trypticase Soy Agar (TSA) because mung beans have a similar nutrient composition to the soybean component of TSA. In addition, to address the problem of the high-cost commercial culture media, the cost of the preparation of Mung Bean Agar (MBA) will be recorded and compared to the local price of TSA. As this study seeks an alternative to nonselective culture media, various biochemical tests will also be performed to confirm the identity of test microorganisms utilized in the study. This study is anchored on the theory of microbial growth curve. Louis Pasteur developed the artificial culture medium as a model of the human body, as indicated by Sandle [20]. The microbial growth curve was defined as a nonequilibrium thermodynamic process. The anchored theory is supported by the principle of pure culture technique, which forms the basis of the procedures.

This study aims to determine the potential of Mung bean (Vigna radiata) as an alternative culture medium for Trypticase Soy Agar.

Specifically, it seeks to Formulate an alternative culture medium using mung bean as the main component of the culture medium; Identify the needed concentration of agar powder to be added in Mung bean to obtain a firm gel consistency comparable to that of the Trypticase Soy Agar; Inoculate ATCC cultures of Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans onto the MBA and TSA by four-quadrant streaking method; Describe the colony morphology of test microorganisms in MBA and TSA with the use of modified Centers for Disease Control and Prevention (CDC) categorized description of colony characterization; Compare the standard absolute growth index of the test microorganisms in the Mung Bean Agar to Trypticase Soy Agar, the control medium; Confirm the identity of the test microorganisms by performing gram stain and various biochemical tests; Compare the total cost of the preparation of Trypticase Soy Agar and Mung Bean Agar; and Recommend Mung Bean Agar as a potential alternative culture medium to Trypticase Soy Agar.

2.0 METHODOLOGY

2.1 Research Design

This study employed an experimental design with a quantitative approach in comparing the absolute growth index and noting the morphology of colonies of the test microorganisms in Mung Bean Agar as a potential alternative to the commercial media, Trypticase Soy Agar.

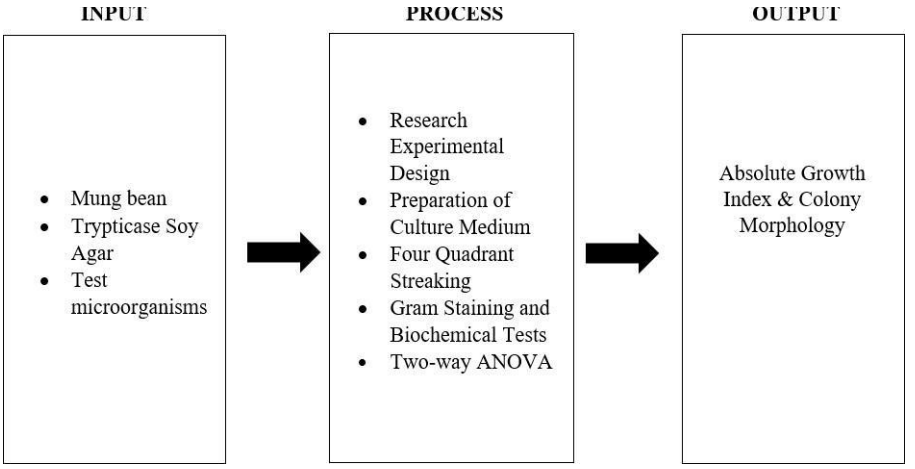

Figure 1: Research Flow Process

2.2 Research Environment

The research was conducted at the University of Cebu-Banilad, 8th floor, Microbiology laboratory, College of Medical Technology. The University of Cebu-Banilad is located along Gov. M. Cuenco Ave, Cebu City, 6000. Moreover, the Mung bean was procured from a farm in Looc Norte, Asturias, Cebu through delivery. The samples were packed in a sterile polyethylene bag and were taken to a taxonomist in the Department of Agriculture (DA), Mandaue City, for authentication.

2.3 Research Subjects

This study utilized purposive convenience as the implemented sampling method. The researchers used Mung beans as they are of the same taxonomic family and similar compositions of soybean in Trypticase Soy Agar. Data were obtained from replicates of the formulated Mung bean culture medium and Trypticase Soy Agar with test microorganisms.

To increase the precision of model parameter estimations, it is a common practice in scientific research to measure each sample unit in triplicates [21] as being the minimum for any inferential statistics [22]. Furthermore, it is valid to compute ANOVA even with just duplicates

in each group in determination of significant difference [23]. As consulted by a statistician, the researchers utilized three replicates of each microorganism in each culture medium, a triplicate of a particular organism in formulated Mung beans culture medium, and another triplicate for the commercial Trypticase soy agar in three trials. A confidence value of 95% with a margin of error of 5% was utilized.

i. **Inclusion Criteria.** The formulation of alternative culture media using a low-cost natural source as a substitute for Trypticase Soy Agar (TSA) was evaluated in this study. Mung beans were bought from a farm in Looc Norte, Asturias, Cebu. Mung beans from Asturias, Cebu, were the only subject for bacteria and fungi culture. The Mung beans that were used are only green seeds. In addition, tested bacteria only include E. coli, S. aureus, and P. aeruginosa. Likewise, the tested fungi include C. albicans. The mentioned tested microorganisms are the only subjects for media culture. The tested bacteria and fungi were provided by the Microbiology laboratory at the University of Cebu-Banilad.

ii. **Exclusion Criteria.** This study does not involve other protein sources for cultivating bacteria and fungi. Mung beans that had sprouted and not green in color were not gathered. The tested microorganisms were not obtained from another laboratory.

2.4 Research Instrument

This section confers the data collection tool that is used in the study. The study utilized the modified Centers for Disease Control and Prevention (CDC)-categorized description of size, surface appearance, color, density, form, and margin in describing the colony morphologies. The Absolute Growth Index (AGI) developed by Mossel et al., was used for quantification. The growth score in each test microorganism in all replicates and trials of Trypticase Soy Agar and Mung Bean Agar was averaged. The microbiology lab provided the necessary tools and supplies for the experiment to be possibly done.

2.5 Data Collection and Treatment

A loopful from ATCC culture of E. coli, S. aureus, P. aeruginosa and C.albicans were inoculated separately to Trypticase Soy Agar using the four-quadrant streaking method. The aseptic method was observed by sterilizing the wire loop over a flame of an alcohol lamp until red hot. The plates were incubated for about 18-24 hours. All glassware and apparatuses were thoroughly cleaned and sterilized using an autoclave. These procedures ensured the creation of sterile media for microbiological experiments.To prepare Mung Bean Agar (MBA) using a mung bean grinder, 1 kg of mung beans was ground into powder and sieved. Researchers mixed 25 g of powdered mung bean and 15 g of agar in 1 L of distilled water, sterilized it in an autoclave, and poured it into petri dishes to solidify. TSA was prepared as per the manufacturer's instructions by suspending 40 g of TSA in 1 L of distilled water, followed by sterilization in an autoclave and pouring into petri dishes. Trypticase Soy Broth (TSB), Pseudomonas Agar Medium (PAM), and Eosin-Methylene Blue Agar (EMB) were also prepared following the respective manufacturer's instructions, sterilized, and dispensed into suitable containers.

The procedures that were performed by the researchers are referred to the set of guidelines made by the Australian Society for Microbiology, whose content is not dissimilar to the standard procedures of Clinical and Laboratory Standards Institute (CLSI) in M22A3 document entitled ‘Quality Assurance for Commercially Prepared Microbiological Culture Media’. The researchers made a Trypticase Soy Broth (TSB) suspension equivalent to 0.5 Mc Farland of E. coli, S. aureus, P. aeruginosa, and C. albicans from the subculture separately. Only one of the researchers performed the streaking using only one wire loop to ensure the uniformity of the technique. With a sterile metal wire loop, one loopful of TSB suspension was streaked on the solid culture media vertically by partially lifting the lid of the petri dish. With utmost caution, the liquid bubble on the loop collapsed in the first streak on

the first quadrant of each plate. The inoculum was then spread horizontally by streaking it back and forth on the first quadrant. The petri dish was turned 90o, and the loop streaked across the first quarter a few times, then over the second quadrant repeatedly. The loop was always meant to initially touch the corner of the preceding quadrant to streak over the following quadrant. There should never be any overlap between the final streaks of any two adjacent quadrants. The same procedure is followed until the fourth quadrant. The researchers then incubated the plates in an inverted position at 37 oC for 24 hours. Such procedure was followed for both Trypticase Soy Agar and Mung Bean Agar. The test microorganisms are described according to its characterization in size, surface appearance, color, density, form, and margin in both Mung Bean Agar and Trypticase Soy Agar. The extent of colonization of each test microorganism along the four quadrants in Mung bean Agar and Trypticase Soy Agar was rated according to the Absolute Growth Index (AGI). Colony morphologies and resulting scores of each growth of certain microorganisms were validated by one microbiologist and one registered medical technologist as recommended by the statistician to ensure accuracy of results. On both ends of the slides, place a drop of normal saline and mix the thin smears of the cultures as mentioned earlier: one for the isolates from Trypticase Soy Agar and one for Mung Bean Agar. Gentian violet solution, a primary stain, should be applied to the prepared smear and left on for one minute before being washed with tap water. Flood the smear with Gram's iodine for a minute, then rinse with tap water. With acetone alcohol, decolorize the smear until the primary stain washes off and color flows off from the slide. Wash with tap water. Safranin as a counterstain, flood for 30 seconds, then wash with tap water. Blot dry and examine under OIO. The tests utilized in the study to confirm the identity of S. aureus in subcultured media, Trypticase Soy Agar and Mung Bean Agar are as follows:

i. Catalase Test

ii. Slide Coagulation Test

iii. Tube Coagulation Test

The tests utilized in the study to confirm the identity of E. coli in subcultured media, Trypticase Soy Agar, and Mung Bean Agar are as follows:

i. Eosin-Methylene Blue

The tests utilized in the study to confirm the identity of P. aeruginosa in subcultured media, Trypticase Soy Agar and Mung Bean Agar are as follows:

i. Pyoverdine Production

The tests utilized in the study to confirm the identity of C. albicans in subcultured media, Trypticase Soy Agar and Mung Bean Agar are as follows:

i. Germ Tube Production

This section presents the statistical tools utilized in the analysis and interpretation of the data to acquire the necessary results in the study. In this study, the following statistical treatments were utilized:

i. Frequency Count and Simple Percentage were used to determine the number of plates that showed a particular colony morphology per the CDC-categorized description of colony characterization.

ii. Summation of the Costs was used to calculate how much it cost overall to prepare Mung Bean Agar and Trypticase Soy Agar. Next, direct comparisons of the prices of each culture media were made.

iii. Two-way ANOVA was utilized to determine the significant difference between the absolute growth index of the formulated Mung Bean Agar and Trypticase Soy Agar. With the use of dependable software like Microsoft Excel, the researchers were able to employ these statistical techniques to provide precise and accurate

data analysis and outcomes. Additionally, a statistical basis of error was used, with an alpha (a) value of 0.05. If the F-critical value is greater than the alpha, then the null hypothesis is rejected, which implies a significant difference between the absolute growth index of Mung beans culture medium and commercial Trypticase soy agar. Otherwise, the researchers were required to accept the null hypothesis.

3.0 RESULTS AND DISCUSSION

3.1 Colony Morphology

Table 1: Colony Morphology of Escherichia coli in Trypticase Soy Agar and Mung Bean Agar

	Mung Bean Agar			Trypticase Soy Agar		
	Morphology	**Frequency**	**%**	**Morphology**	**Frequency**	**%**
Size	pinpoint	9	100%	medium	9	100%
Surface Appearance	mucoid	9	100%	mucoid	9	100%
Color	gray	9	100%	yellow	9	100%
Density	opaque	9	100%	transparent	9	100%
Form	punctiform	9	100%	circular	9	100%
Margin	entire	9	100%	entire	9	100%

Table 1 presents the frequency distribution of the colony morphology of Escherichia coli both in Mung Bean Agar and Trypticase Soy Agar. All the plates in MBA with E. coli showed pinpoint, mucoid, gray, opaque, punctiform, and entire colonies. On the contrary, TSA demonstrated medium, mucoid, yellow, transparent, circular, and entire colonies in all plates.

Table 2 presents the frequency distribution of the colony morphology of the Staphylococcus aureus both in Mung Bean Agar and Trypticase Soy Agar. The replicate plates of MBA with S. aureus displayed pinpoint, smooth, grayish yellow, opaque, punctiform, and entire colonies. Meanwhile, TSA demonstrated small, mucoid, yellow,

transparent, circular, and entire colonies in all plates.

Table 2: Colony Morphology of Staphylococcus aureus in Mung Bean Agar and Trypticase Soy Agar

	Mung Bean Agar			Trypticase Soy Agar		
	Morphology	**Frequency**	**%**	**Morphology**	**Frequency**	**%**
Size	pinpoint	9	100%	small	9	100%
Surface Appearance	smooth	9	100%	mucoid	9	100%
Color	grayish yellow	9	100%	yellow	9	100%
Density	opaque	9	100%	transparent	9	100%
Form	punctiform	9	100%	circular	9	100%
Margin	entire	9	100%	entire	9	100%

Table 3: Colony Morphology of Pseudomonas aeruginosa in Mung Bean Agar and Trypticase Soy Agar

	Mung Bean Agar			Trypticase Soy Agar		
	Morphology	**Frequency**	**%**	**Morphology**	**Frequency**	**%**
Size	pinpoint	9	100%	medium	9	100%
Surface Appearance	smooth	9	100%	rough	9	100%
Color	grayish yellow	9	100%	green	9	100%
Density	opaque	9	100%	transparent	9	100%
Form	punctiform	9	100%	irregular	9	100%
Margin	entire	9	100%	entire	9	100%

Table 3 shows the frequency distribution of the colony morphology of Pseudomonas aeruginosa both in Mung Bean Agar and Trypticase Soy Agar. All nine (9) replicate plates of MBA with P. aeruginosa showed pinpoint, smooth, grayish yellow, opaque, punctiform, and entire colonies. The researchers also noted the color of the MBA culture media with P. aeruginosa colonies were changed from gray into yellow

comparable to that of the Trypticase Soy Agar after 24 hours of incubation. On the other hand, TSA displayed medium, rough, green, transparent, irregular, and entire colonies on all plates.

Similarly, Shareef found that Pseudomonas aeruginosa exhibited prominent characteristics when grown on formulated culture media made of legumes [13]. These characteristics included the formation of large, irregularly shaped colonies with an opaque appearance, accompanied by a sweet odor. These colonies were further distinguished by distinctive pigments, primarily the blue-colored pyocyanin, which spread throughout the culture media.

Table 4: Colony Morphology of Candida albicans in Mung Bean Agar and Trypticase Soy Agar

	Mung Bean Agar			Trypticase Soy Agar		
	Morphology	**Frequency**	**%**	**Morphology**	**Frequency**	**%**
Size	pinpoint	9	100%	small	9	100%
Surface Appearance	smooth	9	100%	mucoid	9	100%
Color	grayish white	9	100%	yellow	9	100%
Density	opaque	9	100%	transparent	9	100%
Form	punctiform	9	100%	circular	9	100%
Margin	entire	9	100%	entire	9	100%

Table 4 shows the frequency distribution of the colony morphology of the Candida albicans both in Mung Bean Agar and Trypticase Soy Agar. All nine (9) replicate plates of MBA with C. albicans showed pinpoint, smooth, grayish-white, opaque, punctiform, and entire colonies. On the other hand, TSA displayed small, mucoid, yellow, transparent, circular, and entire colonies on all plates.

According to the study by Shareef, the colonies of fungi in formulated alternative culture medium, made out of legumes, are smaller in size

as compared to their growth on commercial Potato Dextrose Agar [13]. Additionally, the color of the bacterial colonies was also noted to conform to the color of the formulated culture medium [13].

3.2 Absolute Growth Index

The extent of colonization of the test microorganisms was determined using the four-quadrant streaking method and was expressed as an absolute growth index.

Table 5: Comparison between the of Absolute Growth Index of Test Microorganisms in Mung Bean Agar and Trypticase Soy Agar

Source	Sum of Squares	df	Mean of Squares	F-Value	F-Critical Value	P-Value
Between	1.7882	3	0.5961	1.3651	2.7482	0.2614
Within	27.9444	64	0.4366			
Total	29.7326	67				

Statistically Significant at 0.05 levels

A two-way ANOVA was utilized to compare the absolute growth index of test microorganisms between Mung Bean Agar and Trypticase Soy Agar. The total mean AGI of 3.125 with a standard deviation of 0.75 in MBA is much closer to that of TSA's total mean AGI of 3.25 with a standard deviation of 0.58. Table 5 showed that there is no statistically significant difference between the absolute growth index of TSA and MBA given with the two conditions, the F-value of 1.3651 is lesser than the critical value of 2.7482, and the P-value of 0.2614 is greater than 0.05 level of significance. Given the resulting data presented, the null hypothesis stands accepted. It infers that the mean absolute growth index of test microorganisms in both MBA and TSA does not vary that much. Hence, the environment provided by the MBA for the test microorganisms is comparable to that of TSA in terms of absolute growth index.

As a validation, the study of Arulanantham et al. presents that all the formulated media that utilized legumes as protein sources supported the growth of all test organisms, namely *Bacillus sp.*, *Klebsiella sp.*, *Pseudomonas sp.*, *Staphylococcus sp.*, and *Escherichia coli*. Although *Klebsiella sp.* demonstrated the least growth among the formulated media, the growth of other test organisms, including *Pseudomonas sp.*, *Staphylococcus sp.*, and *E. coli* showed no significant difference when compared to the growth in Nutrient Agar that is utilized as a positive control in the said study [14]. Furthermore, the ability of mung beans to support the development of fungi stands in line with the study of Ilmi et al., which uses mung bean sprouts as an alternative fungal growth medium. The collected results of the researchers of the said study pointed out that the mung bean sprout medium gave a similar growth performance of *Saccharomyces cervesiae, Kluyveromyces marxianus, Aspergillus oryzae* and *Trichoderma viridae* to that of commercial media, which is Potato Dextrose Agar and Malt Extract Agar, in particular [24].

3.3 Cost of the Culture Media

Table 6: Economical Comparison of Culture Media

Cost in ₱/500g	
Trypticase Soy Agar	**Mung Bean Agar**
P3,600.00	P2,564.375

The cost of Mung Bean Agar was initially obtained from the grams of mung bean and agar powder used in the preparation and equated into 500 grams. The calculated price per gram of mung bean (₱0.07/kg) and agar (₱13/g) was then multiplied by the grams used and summed up. On the contrary, the commercial culture media, TSA, was simply recorded by its local price. Table 7 explicitly shows that the preparation of an MBA is less expensive than TSA by approximately ₱1035.63.

Correspondingly, a study in Sri Lanka stated that the formulated alternative culture medium out of legumes costs approximately 300 LKR, or 51 pesos to prepare 1L of the medium while it costs around 400 LKR, or 68.42 pesos, to prepare the commercial Potato Dextrose Agar of the same volume [1]. As a result, the researcher concluded that the said study is feasible and less expensive to use different alternative formulations as culture media in laboratories with basic facilities.

4.0 CONCLUSION

The creation of cost-effective and high-quality cultural media presents huge possibilities for scientific research. The expanding number of studies demonstrating the effectiveness of alternative cultural media implies that they can be just as effective, if not more so, than traditional culture media. Through adopting alternative cultural media, researchers may explore new possibilities and attain comparable or even superior outcomes in their studies [24]. Based on the study's findings, Mung Bean Agar can be used as a potential alternative culture medium for Trypticase Soy Agar. Although most of the colonies in the MBA were pinpoint in size and punctiform, the absolute growth index of the test microorganisms was close to that of TSA. As a result, MBA can provide acceptable environmental conditions for the test microorganisms that have been shown to grow in TSA. Furthermore, the preparation of MBA was also feasible and less expensive compared to that of TSA.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare and are in agreement with the contents of the manuscript.

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