Malays. Appl. Biol. (2022) 51(4): 67-74
https://doi.org/10.55230/mabjournal.v51i4.12

SOYBEAN AS AN ALTERNATIVE NUTRIENT MEDIUM FOR *Bacillus subtilis* GROWTH

NURUL HANISAH ABDUL MALIK, KHANOM SIMARANI and MUHAMAD AFIQ AZIZ*

Institute of Biological Sciences, Faculty of Science, Universiti Malaya,
50603 Kuala Lumpur, Malaysia
**E-mail: afiqaziz@um.edu.my*

Accepted 3 October 2022, Published online 31 October 2022

ABSTRACT

Nutrient agar is a commonly used medium for the isolation and growth of a broad range of microorganisms. The feasibility of using soybean as a base medium in the development of alternative growth media was assessed in this study. Nutrient agar was used as a standard guide to evaluating the performance of the formulated soybean agar. *Bacillus subtilis* was inoculated and allowed to grow on nutrient agar and soybean agar. Their growth was compared within 24 h after inoculation based on the morphology of individual colonies formed on both media and the pattern of bacterial growth. Our results showed that soybean agar had comparable performance to nutrient agar as the morphological characteristics of *B. subtilis* colonies formed on both media are generally identical in terms of texture, margin, optical properties, colour, elevation, and shape. However, due to the similar appearance of the bacterial colonies and the soybean agar, the colonies formed on the soybean agar were slightly larger than those formed on nutrient agar. In addition, our findings also revealed that agar strips formed the best soybean agar compared to gelatin and agar powder. Ultimately, this study has shown that locally available soybeans and agar strips can be easily formulated as an alternative to commercial nutrient agar and have great potential for bacteriological research.

Key words: Alternative, *Bacillus subtilis*, growth, soybean, nutrient agar

INTRODUCTION

In the field of microbiology, the study of microorganisms depends on a substantial extent of the ability to isolate the organism of interest and grow it alive under specific laboratory conditions. The cultivation of microorganisms can be challenging due to their specific environmental and nutritional requirements (Lagier *et al*., 2015). There are various commercially available culture media that support the growth of a wide range of microorganisms. A prime example of a general-purpose medium is nutrient agar (NA). NA is the standard medium widely used for the cultivation of non-fastidious microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Klebsiella pneumoniae* (Neal, 2019). It contains nutrients and energy sources to promote microbial growth and produce identical colonies. Diverse types of microbes produce distinctive characteristics of colonies, such as shape, colour, texture, and edge, which help microbiologists distinguish different types of microbes in a mixed culture.

In recent years, numerous studies have focused on identifying alternative sources of growth media. The most common ingredient is fruit waste, especially fruit peels. In Indonesia, a study was conducted by a group of researchers that focused on the cultivation of *E. coli* using growth media from dragon fruit peels (Putri *et al*., 2018). Pineapple and green banana peels were also used as alternative growth media for fungal cultivation (Anbu *et al*., 2017). A recent study by Hasanin and Hashem showed that watermelon peel dextrose agar was as efficient as conventional media for fungal growth (Hasanin & Hashem, 2020).

Fruit peel-based media were also found to be beneficial for microbial production of bio-cellulose (Kumbhar *et al*., 2015), biodiesel (Carota *et al*., 2020), antimicrobials (Okpalauwaekwe *et al*., 2020), polyhydroxybutyrate (Valdez-Calderón *et al*., 2020), amylase (Iram *et al*., 2021), biosurfactant (Vieira *et al*., 2021) and single cell protein (Haddish, 2015; Umesh *et al*., 2019). In addition to fruit peels, fresh vegetables and vegetable wastes, such as beetroots (Al-Azzauy & Hassan, 2011; Mohammed *et al*., 2018; Borah *et al*., 2020), tomatoes, carrots, cabbage, pumpkin (Deivanayaki & Iruthayaraj, 2012), potato peels, cauliflower stems, and fenugreek stems (Jadhav *et al*., 2018) were also used as major food sources for bacterial and fungal growth media.

Legumes have also been manipulated for the development of new media (Ravimannan *et al*.,

* To whom correspondence should be addressed

2014; Uthayasooriyan *et al*., 2016; Shareef, 2019; Mohammed *et al*., 2020). Legumes are food crops belonging to the Leguminosae family. They are also called grain legumes as they are mainly grown for their edible seeds (Iqbal *et al*., 2006). Some examples of legumes are soybean (*Glycine max*), green bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), groundnut (*Arachis hypogaea*), lentil (*Lens culinaris),* mung bean (*Vigna radiata*) and broad bean (*Vicia faba*) (Maphosa & Jideani, 2017).

The soybean, also known as the soja bean or soya bean, is the most economically useful bean in the world and was cultivated in central China as early as 7000 BC (Deasy, 1939). Soybeans are one of the most nutritious food at a very affordable price. 100 g of boiled soybeans contain about 16.6 g of protein, 9.9 g of carbohydrate, 9.0 g of fat, 6.0 g of fibre, and 3.0 g of sugar (Arnarson, 2019). This legume has been included in the human diet as a healthier substitute for animal protein due to its high protein content (Joshi & Kumar, 2015; Arnarson, 2019). Soybeans contain about 38–45% protein content, which is higher compared to raw nuts (7–26%) and cereals (8–15%), making them an excellent source of protein for microbial growth (Liu, 1997; Brufau *et al*., 2006; Kouris-Blazos & Belski, 2016).

Gelling agents, also known as solidifying agents, are vital in the formulation of culture media as they convert liquid culture medium into semi-solid or solid. They provide strength and stability to the medium, whereas, the concentration of gelling agents added affects the diffusion properties of the medium (Palaniraj & Jayaraman, 2011; Das *et al*., 2015). Gelatin and agar are the first generations of gelling agents used in the production of growth media. Gelatin was first used in the development of growth media by Robert Koch in 1881(Sandle, 2011; Basu *et al*., 2015). A year later, agar derived from *Gracilaria* sp. was developed and since then, this agent has often been used as an alternative to gelatin for the preparation of solid cultures in microbiological research (Das *et al*., 2015). Food-grade agar and bacteriological grade agar are the two most common forms of agar. Commercial bacteriological agar is usually more expensive than food-grade agar. Nevertheless, the solidification and stability properties of food-grade agar are equivalent to those of bacteriological agar (Hanond *et al*., 2007; Petrovski & Tillett, 2012; Das *et al*., 2015).

In this study, our main objective is to develop a low-cost culture medium for bacterial growth using soybean as the primary source of nutrients. Instead of bacteriological agar, food-grade gelatin and agar were purchased from local markets to be used as gelling agents for the formulation of soybean agar. The performance of the newly formulated soybean agar was evaluated by comparing the growth and morphology of *Bacillus subtilis* in both standard nutrient media and soybean agar.

MATERIALS AND METHODS

Sample collection

Soybeans (*Glycine max*) were selected as the nutrient source for alternative nutrient agar. Samples were obtained from the local supermarket and ground using an electric blender (Panasonic MX800S) and were filtered through a sieve twice to remove any lumps or large particles that remained in the soy powder. The powder was stored dry in an airtight container until further use.

Test microorganism

The bacteria used in the study were *Bacillus subtilis*. The organism was obtained from Bendosen Laboratory Chemicals (Bendosen). To prepare a stock culture, an amount of a lyophilized *B. subtilis* culture was inoculated into 1 mL of distilled water until the clear water became turbid. The liquid bacterial inoculum was then spotted onto commercial nutrient agar (Hudson & Bendosen) to obtain individual bacterial colonies. The plates were then incubated for 24 h at room temperature.

Formulation of soybean agar

Three ingredients were used to prepare the media: Soybean powder, sterile distilled water and solidifying agents. Three types of locally available solidifying agents (gelatin, agar powder & agar strips) were used instead of commercial agar-agar. Different SBA formulations are listed in Table 1. For each formulation, the amount of soybean powder and sterile distilled water applied was standardized at 3.0 g and 100 mL, respectively. The mass required for gelatin, agar powder and agar strips were determined according to the manufacturers' instructions, which were 28.50 g, 22.50 g and 1.57 g, respectively. After measuring, the ingredients were mixed and boiled together. The medium was then covered with aluminium foil before being dry-heat sterilised using a drying oven (S Business Series™) at 121 °C for 10-15 min. Apart from sterilisation, this step was important to further dissolve the solidifying agents that had not been completely dissolved during the boiling process. Generally, the moist heat sterilisation method using an autoclave system is the most effective method for sterilising culture media as it is capable of killing all types of microorganisms, including spores. As there is no accessible autoclave system in our laboratory, we could only perform dry-heat sterilisation to sterilise all the media prepared. The dry-heat technique, in general, necessarily involves a prolonged exposure (1 to 3 h) to a high temperature (140 to 170 °C) to kill the microorganisms effectively (Darmady *et al*., 1961). Such settings, however, may be impractical for media sterilisation as media overheating can lead to a decline in media stability and bacterial performances, as well as media darkening, precipitation and pH drift

(Sandle, 2017). Hence, we decided to standardise the sterilisation temperature and time for the dry-heat method in our study, similar to the standard protocol for the moist-heat method, to reduce media overheating during sterilisation. After sterilisation, the foil was removed and the media was cooled until it reached 40-45 °C. The media was then carefully poured into sterile Petri dishes and was allowed to solidify completely at room temperature. The solidification time for each SBA formulation was recorded. The time required for each formulation to fully solidify was optimised from 35 – 60 min (Figure 1). The colour and appearance of the media formed were also observed and recorded. The best SBA agar formed was selected for the evaluation test with commercial nutrient agar (NA).

Microbial inoculation of the media

For microbial inoculation, two streaking techniques, four-quadrant and continuous, were used to evaluate and compare the performance of NA and SBA. Individual colonies from the stock culture were streaked on both media using sterilised nichrome wire loops. To reduce the risk of contamination, the plates were then covered with parafilm and incubated at room temperature (23-26 °C) for 24 h. After incubation, the morphology of the colonies formed and the growth pattern of the bacteria on both media was observed and recorded.

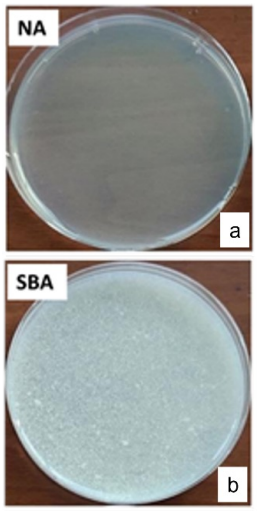
RESULTS

Comparison of solidifying agents for soybean agar media

In this study, the SBA formulation mainly consists of three main ingredients, namely soybean powder, sterile distilled water, and a solidifying agent. Three types of solidifying agents were used in this formulation, namely agar powder, gelatin, and agar strips. Our results showed that agar strips formed the best SBA media compared to agar powder and gelatin. We found that the texture and firmness of the agar strips-based SBA were better compared to other gelling agents. Moreover, only 1.57 g of agar strips in 100 mL of distilled water was required, which is 20 times less than the amount needed for

Table 1. Formulations of soybean agar

Formulation	Soybean powder (g)	Sterile distilled water (mL)	Solidifying agents (g)		
			Gelatin	Agar powder	Agar strips
1	3.0	100	28.5		
2	3.0	100		22.5	
3	3.0	100			1.57



Characteristics	Nutrient agar (NA)	Soybean agar (SBA) – 3.0g		
		Gelatin	Agar powder	Agar strips
Mass used per 100ml distilled water (g)	0.28	28.5	22.5	1.57
Time to solidify (min)	36	>60 (Partially solid)	40 – 50 (Partially solid)	35 - 45
Firmness	Firm	Not firm	Not firm	Firm
Turbidity	Clear	Slightly turbid		
Particle	No	Yes		
Colour	Light amber	Light ivory		

Fig. 1. Visual inspection of commercial nutrient agar (NA) and soybean agar (SBA). NA showed clear and transparent features (a), while agar strip-based SBA showed white and opaque features (b). NA and SBA were prepared in 100 mL distilled water using 0.28 g commercial nutrient powder and 3.0 g soybean powder, respectively. Both media were incubated at room temperature for 24 h before the visual inspection. The properties of SBA using different solidifying agents were compared with commercial NA and summarised in the table (right).

gelatin (28.5 g) and agar powder (22.5 g), as shown in Figure 1. More importantly, the SBA media was observed to take about 35 – 45 min to fully set, similar to the setting time for NA (36 min). In contrast, the agar powder-based SBA was partially solidified even after more than 40 min of incubation. To accelerate the solidification time of the agar powder-based SBA, we decided to incubate the agar powder-based SBA at 4 °C. Our observation suggested that the agar powder-based SBA took one hour to solidify at lower temperatures but is not very stable as the SBA plate was partially solidified after a few hours at room temperature (23-26 °C). A similar result was obtained for gelatin-based SBA. Compared to other solidifying agents, the gelatin-based SBA needed at least 60 min to solidify, which was almost twice as long as the commercial culture medium (Figure 1). In terms of physical appearance, the commercial NA looked clear and transparent, while the SBA was slightly cloudy due to the small bean particles in the medium (Figure 1). We also found that the colour of NA and SBA was light amber and light ivory, respectively (Figure 1).

Growth of *Bacillus subtilis* on different media

Next, we examined the functionality of SBA by observing the differences in the growth of *B. subtilis* in SBA and NA that were incubated at room temperatures (23-26 °C). Based on our observations, there was no substantial difference between NA and SBA in terms of the number of colonies formed on the agar (Figure 2). We also noticed that the morphology of the bacterial colonies on both media was comparable, as summarized in Table 2. Our visual inspection of two different streaking techniques showed identical growth patterns of *B. subtilis* colonies, suggesting that SBA can support the growth of the microorganisms of interest similar to commercial agar (Figure 2).

DISCUSSION

The global pandemic has had a major impact on the development of research around the world. Researchers are constantly looking for various feasible initiatives to minimise their research expenditures. This includes the development of novel media made from low-cost, readily available materials. In this study, we used three different solidifying agents (gelatin, agar powder, and agar strips) that are widely available in the local Asian market. Gelatin was one of the first gelling agents used in various growth media and was derived from the connective tissue of animals (Ledward, 2000). Agar, on the other hand, is a plant-based gelling agent that is usually derived from algae, mainly *Gelidium* sp., *Gracillaria* sp., and *Pterocladia* sp. (Das *et al*., 2015). Our results showed that gelatin has the poorest gelling effect among the firming agents. Previous studies have shown that the gelling effect of this agent was achieved when the media temperature was less than 20 °C (Mad-Ali *et al*., 2017). When gelatin-based media are incubated at 37 °C, they melt easily, affecting the stability of the media, which is critical for bacterial growth (Das *et al*., 2015; Bonnet *et al*., 2020). In addition, digestion and degradation of gelatin can occur in the presence of an enzyme known as gelatinase, which is commonly found in certain microbial species, such as *Pseudomonas aeruginosa*, *Serratia marcescens,* and *Bacillus* sp. (Sandle, 2011; Balan *et al*., 2012; Bonnet *et al*., 2020).

In addition, we have found that agar, especially agar strips, makes a better SBA medium than gelatin. The use of agar in media formulation has several advantages. First of all, agar is relatively stable over a wide range of temperatures. The average solidification temperature of conventional agar is between 32 and 42 °C, and its melting temperature is around 85 °C (Das *et al*., 2015). Moreover, the strength of the medium can be easily changed by adjusting the amount of agar used (Raina & Babbar, 2011). Apart from its transparent properties, it is also metabolically inert, so it does not affect the microbial biochemical processes that play an important role in their growth in the medium (Petrovski & Tillett, 2012).

In previous studies using soybean and soy-based products for media preparation, most researchers considered several parameters in media preparation, especially pH, which may affect microbial growth in the media (Ravimannan & Pathmanathan, 2016; Uthayasooriyan *et al*., 2016; Shareef, 2019). Nevertheless, in the present study, we disregarded the influence of pH on microbial growth to significantly reduce the risk of contamination. Furthermore, due to the lack of suitable sterilisation equipment in our laboratory, such as an autoclave, we restricted the use of a conventional pH meter to measure the final pH of the SBA media, as this could contribute to the contamination of the media.

The potential use of soybeans as a cheap source of nutrients for alternative growth media is undeniable. Soybeans contain nine essential amino acids such as valine, tryptophan, threonine, phenylalanine, methionine, lysine, leucine, isoleucine, and histidine (Monte Singer *et al*., 2020). Previous studies have shown that soy-based products can serve as good sources of nutrients for the cultivation of various microorganisms, especially bacteria and fungi (Ravimannan & Pathmanathan, 2016; Uthayasooriyan *et al*., 2016; Shareef, 2019; Cruz *et al*., 2020). Similar results were obtained in this study, where *B. subtilis* grew very well in both media, SBA and NA. More importantly, the morphology of the bacterial colonies formed on both culture media was very identical in terms of texture, margin, optical properties, colour, height, and shape. Nevertheless, colonies formed on SBA were slightly larger compared to NA. We

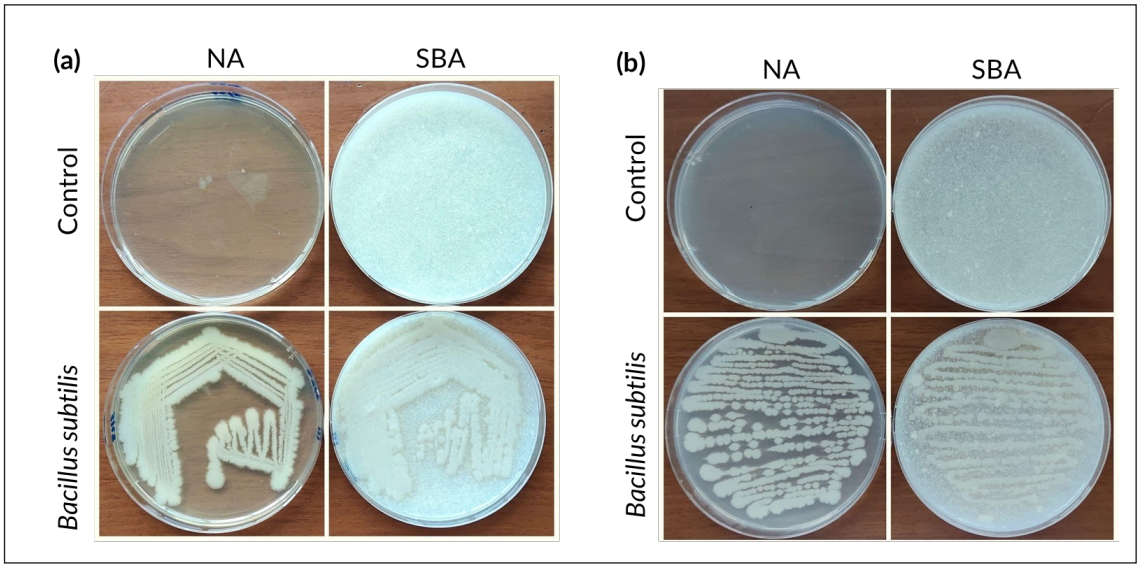


Fig. 2. Comparison of *B. subtilis* growth in NA and SBA that were inoculated using four-quadrant (a) and continuous (b) streaking techniques. Inoculated media were incubated for 24 h at room temperature (23–26 °C). The agar plates without *B. subtilis* represent negative control to ensure no bacterial contamination.

Table 2. The morphology of *B. subtilis* in NA and SBA. The bacterial colonies formed on SBA and NA were compared visually

Colony morphology	Nutrient Agar	Soybean Agar
Texture	Rough	Rough
Margin	Jagged edges	Erose
Optical property	Opaque	Opaque
Colour	Slightly yellow	Yellowish white
Colonies size	Intermediate	Slightly bigger
Form	Fuzzy	Irregular, slightly fuzzy
Elevation	Flat	Flat

suspected that this was most likely due to the white and granular appearance of SBA, which made the white *B. subtilis* colonies appear larger. It was also difficult to examine the bacterial colonies that formed on SBA compared to the clear, transparent NA. A similar problem was reported by other researchers who claimed that visual inspection of bacterial colonies on soy flour-based media was a time-consuming process because white bacterial colonies on the soy-based media were indistinguishable, making colony counting difficult (Mosher *et al*., 2009).

Even though *B. subtilis* grew successfully on SBA, more future research into the medium's performance in microbial cultivation is required, along with a few recommendations to guarantee the medium's optimal performance. The first suggestion is to utilise contemporary laboratory facilities to refine and improve SBA formulation. These facilities may help users to produce finer soybean powder, reducing the difficulty of visual inspection of microbial colonies on SBA. Furthermore, employing modern equipment, such as a pH meter and an autoclave system, which was not accessible in our present study, could provide a more proper and reliable analysis of SBA's efficacy in microbial cultivation and other microbiological tests. Next, various bacterial and fungal species should be tested on SBA to determine their compatibility with the medium, as the SBA's feasibility in the study was confined to a single bacterial species (*Bacillus* sp.). Then, quantitative assessments should be conducted to examine and compare the performance of SBA and standard NA in cultivating these microbial species, permitting a more comprehensive and accurate analysis of SBA performance.

CONCLUSION

This present study has succeeded in designing a novel culture medium by exploiting locally available and cheap ingredients like soybean (as a nutrient source) and agar strips (as a solidifying agent). The newly formulated SBA was capable of sustaining *B. subtilis* growth and had equivalent performance to NA. On top of that, our findings have shown that agar strips

form the best SBA medium compared to the other two solidifying agents tested (gelatin and agar powders). Thus, food-grade agar strips have the potential to be a low-cost alternative to commercial bacteriological agar, which is extensively used in media preparation. Despite this, there are several shortcomings in our research that may obscure the actual findings. One of the difficulties we encountered was observing the microbial colonies formed on SBA due to their similar appearance to SBA. Furthermore, we were only able to conduct a qualitative study that is less accurate than a quantitative analysis due to the absence of modern laboratory equipment. Therefore, additional qualitative research incorporating sophisticated laboratory equipment and a vast number of microbes is highly recommended to fully explore the SBA's functionality in microbial cultivation and other microbiological analysis.

ACKNOWLEDGEMENTS

We would like to thank SMK Bukit Assek, Sibu, Sarawak, especially Mr Kandou Ngadi, Mrs Asmah Bujang and Mrs Cecelia Guwang, for allowing us to utilize their laboratory and equipment throughout this project. Also, we wish to extend our appreciation to the Institute of Biological Sciences, the Faculty of Science, and the University of Malaya for allowing us to do this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Al-Azzauy, A.A.M. & Hassan, A.M. 2011. The beetroot juice as a bacterial growth and maintenance medium for many pathogenic bacteria. *Iraqi Journal of Market Research and Consumer Protection*, **3(5)**: 147–161.

Anbu, S., Saranraj, P., Padma, J. & Punithavalli, K. 2017. Fruits peel waste as a novel media for the growth of economically important Fungi. *Journal of Pharmacognosy and Phytochemistry*, **6(6)**: 426–428.

Arnarson,A. 2019. Soybeans 101:Nutrition Facts and Health Effects [WWW Document]. URL https://www.healthline.com/nutrition/foods/soybeans#plant-compounds (accessed 11.6.21).

Balan, S.S., Nethaji, R., Sankar, S. & Jayalakshmi, S. 2012. Production of gelatinase enzyme from Bacillus spp isolated from the sediment sample of Porto Novo Coastal sites. *Asian Pacific Journal of Tropical Biomedicine*, **2(3)**: S1811–S1816. https://doi.org/10.1016/S2221-1691(12)60500-0

Basu, S., Bose, C., Ojha, N., Das, N., Das, J., Pal, M. & Khurana, S. 2015. Evolution of bacterial and fungal growth media. *Bioinformation*, **11(4)**: 182–184. https://doi.org/10.6026/97320630011182

Bonnet, M., Lagier, J.C., Raoult, D. & Khelaifia, S. 2020. Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. *New Microbes and New Infections*, **34**: 100622. https://doi.org/10.1016/j.nmni.2019.100622

Borah, A., Mahanta, J. & Narzary, D. 2020. Formulation of cost-effective alternative fungal culture media using local resources. *Journal of Advanced Plant Sciences*, **10(2)**: 31–36.

Brufau, G., Boatella, J. & Rafecas, M. 2006. Nuts: source of energy and macronutrients. *British Journal of Nutrition*, **96(S2)**: S24–S28. https://doi.org/10.1017/BJN20061860

Carota, E., Petruccioli, M., D'Annibale, A., Gallo, A.M. & Crognale, S. 2020. Orange peel waste–based liquid medium for biodiesel production by oleaginous yeasts. *Applied Microbiology and Biotechnology*, **104(10)**: 4617–4628. https://doi.org/10.1007/s00253-020-10579-y

Cruz, C.H. da S., Santos, J.B. dos, Santos, F.P. dos, Silva, G.M.M., Cruz, E.F.N. da, Ramos, G.L. de P.A. & Nascimento, J. dos S. 2020. Texturized soy protein as an alternative low-cost media for bacteria cultivation. *Bacterial Empire*, **3(4)**: 74–76. https://doi.org/10.36547/be.2020.3.4.74-76

Darmady, E.M., Hughes, K.E.A., Jones, J.D., Prince, D. & Tuke, W. 1961. Sterilization by dry heat. *Journal of Clinical Pathology*, **14(1)**: 38–44. https://doi.org/doi.org/10.1136/jcp.14.1.38

Das, N., Triparthi, N., Basu, S., Bose,C., Maitra, S. & Khurana,S. 2015. Progress in the development of gelling agents for improved culturability of microorganisms. *Frontiers in Microbiology*, **6**: 698. https://doi.org/10.3389/fmicb.2015.00698

Deasy, G.F. 1939. The Soya Bean in Manchuria'. Economic Geography, 15(3): 303–310.

Deivanayaki, M. & Iruthayaraj, A.P. 2012. Alternative vegetable nutrient source for microbial growth. *International Journal of Biosciences*, **2(5)**: 47–51.

Haddish, K. 2015. Production of single cell protein from fruit of beles (Opuntia ficus-Indica L.) peels using saccharomyces cerevisiae. *Journal of Microbiology & Experimentation*, **2(7)**: 73. https://doi.org/10.15406/jmen.2015.02.00073

Hanond, P., Samerpitak, K., Kongthawon, A. & Thrakarathai, K. 2007. Application of food grade agar for preparation as media using in microbiological laboratory learning. *Srinagarind Medical Journal*, **22(4)**: 376–383.

Hasanin, M.S. & Hashem, A.H. 2020. Eco-friendly, economic fungal universal medium from watermelon peel waste. *Journal of*

Microbiological Methods, **168**: 105802. https://doi.org/10.1016/j.mimet.2019.105802

Iqbal, A., Khalil, I.A., Ateeq, N. & Sayyar Khan, M. 2006. Nutritional quality of important food legumes. *Food Chemistry*, **97(2)**: 331–335. https://doi.org/10.1016/j.foodchem.2005.05.011

Iram, N., Shakir, H.A., Irfan, M., Khan, M., Ali, S., Anwer, A., Saeed, S. & Qazi, J.I. 2021. Statistical optimization of amylase production using grape fruit peels in submerged fermentation. *Acta Scientiarum-Technology*, **43**: 1–11. https://doi.org/10.4025/actascitechnol.v43i1.50538

Jadhav, P., Sonne, M., Kadam, A., Patil, S., Dahigaonkar, K. & Oberoi, J.K. 2018. Formulation of cost effective alternative bacterial culture media using fruit and vegetables waste. *International Journal of Current Research and Review*, **10**: 2. https://doi.org/10.7324/ijcrr.2018.1022

Joshi,V. & Kumar,S. 2015. Meat analogues: Plant based alternatives to meat products-A review. *International Journal of Food and Fermentation Technology*, **5(2)**: 107. https://doi.org/10.5958/2277-9396.2016.00001.5

Kouris-Blazos, A. & Belski, R. 2016. Health benefits of legumes and pulses with a focus on Australian sweet lupins. *Asia Pacific Journal of Clinical Nutrition*, **25(1)**: 1–17. https://doi.org/10.6133/apjcn.2016.25.1.23

Kumbhar, J.V., Rajwade, J.M. & Paknikar, K.M. 2015. Fruit peels support higher yield and superior quality bacterial cellulose production. *Applied Microbiology and Biotechnology*, **99(16)**: 6677–6691. https://doi.org/10.1007/s00253-015-6644-8

Lagier, J.C., Edouard, S., Pagnier, I., Mediannikov, O., Drancourt, M. & Raoult, D. 2015. Current and past strategies for bacterial culture in clinical microbiology. *Clinical Microbiology Reviews*, **28(1)**: 208–236. https://doi.org/10.1128/CMR.00110-14

Ledward, D.A. 2000. Gelatin. In: *Handbook of hydrocolloids*. G.O. Phillips and P.A. Williams (Eds.). Woodhead Publishing Ltd. pp. 67–86.

Liu, K. 1997. Chemistry and nutritional value of soybean components. In: *Soybeans*. Springer, Boston, MA. pp. 25–113. https://doi.org/10.1007/978-1-4615-1763-4_2

Mad-Ali, S., Benjakul, S., Prodpran,T. & Maqsood, S. 2017. Characteristics and gelling properties of gelatin from goat skin as affected by drying methods. *Journal of Food Science and Technology*, **54(6)**: 1646–1654.

Maphosa, Y. & Jideani, V.A. 2017. The Role of Legumes in Human Nutrition. In: Functional Food - Improve Health through Adequate Food. Intech London. pp. 13. https://doi.org/10.5772/intechopen.69127

Mohammed, A.A., Al-Mugdadi, S.F.H., Al-Sudani, B.T. & Kamel, J.K. 2018. Beetroot juice as an alternative growth and maintenance medium for six isolates of mycobacterium spp. *Journal of Pharmaceutical Sciences and Research*, **10(8)**: 1881–1884.

Mohammed, B.B., Shatti, Z.O., Jasim, E.I., Dari,W.A. & Nasam Alfraji, M.D. 2020. Local culture medium from the legumes mixture as a novel media for the growth and stimulation of prodigiosin pigment which production from Serratia marcescens that isolated environmentally. *Plant Archives*, **20(1)**: 991–1000.

Monte Singer, W., Zhang, B., Rouf Mian, M.A. & Huang, H. 2020. Soybean Amino acids in health, genetics, and evaluation. In: *Soybean for Human Consumption and Animal Feed*. IntechOpen. pp. 14. https://doi.org/10.5772/intechopen.89497

Mosher, R., Crawford, K. & Lukowiak, A. 2009. A soy-based alternative to traditional bacterial nutrient media. *American Biology Teacher*, **71(1)**: 49–51. https://doi.org/10.1662/005.071.0111

Neal, E. 2019. Organisms That grow on a nutrient agar plate [WWW Document]. Sciencing. URL https://sciencing.com/organisms-grow-nutrient-agar-plate-8094992.html (accessed 30.5.21).

Okpalauwaekwe,E.O.,Umedum,C.U.,Iheukwumere, I .H. & Akakuru, L.C. 2020. Optimization of mycological media using agro waste for the production of antimicrobial substance. *Microbiology Research Journal International* , **30(8)**: 67–73. https://doi.org/10.9734/mrji/2020/v30i830252

Palaniraj,A. & Jayaraman,V. 2011. Production, recovery and applications of xanthan gum by Xanthomonas campestris. *Journal of Food Engineering*, **106(1)**: 1–12. https://doi.org/10.1016/j.jfoodeng.2011.03.035

Petrovski,S. & Tillett,D. 2012. Back to the kitchen: Food-grade agar is a low-cost alternative to bacteriological agar. *Analytical Biochemistry*, **429(2)**: 140–141. https://doi.org/10.1016/j.ab.2012.07.011

Putri,C.H., Janica,L., Jannah,M. & Ariana,P.P. 2018. Utilization of Dragon Fruit Waste as Microbial Growth Media, in: Proceeding of the 10th SISAK, Daejeon, Korea, pp. 91–95.

Raina, R.J. & Babbar, S.B. 2011. Evaluation of Blends of Alternative Gelling Agents with Agar and Development of Xanthagar, A Gelling Mix, Suitable for Plant Tissue Culture Media. *Asian Journal of Biotechnology*, **3(2)**: 153–164. https://doi.org/10.3923/ajbkr.2011.153.164

Ravimannan, N., Arulanantham, R., Pathmanathan, S. & Niranjan, K. 2014. Alternative culture media for fungal growth using different formulation of protein sources. *Annals of Biological Research*, **5(1)**: 36–39.

Ravimannan, N. & Pathmanathan, S. 2016. Soy flour as alternative culture media for yeasts.

Global Journal of Science Frontier Research: C Biological Science, **16(3)**: 75–78.

Sandle, T. 2011. History and development of microbiological culture media. *Journal of Institute of Science and Technology* , 10–14.

Sandle, T. 2017. Sterilization of Microbiological Culture Media. *Journal of GxP Compliance*, **21(5)**: 13–22.

Shareef,S. 2019. Formulation of Alternative Culture Media from Natural Plant Protein Sources for Cultivation of Different Bacteria and Fungi. *Zanco Journal of Pure and Applied Sciences*, **31(4)**: 61-69. https://doi.org/10.21271/zjpas.31.4.7

Umesh, M., Thazeem, B. & Preethi, K. 2019. Valorization of pineapple peels through single cell protein production using saccharomyces cerevisiae NCDC 364. *Applied Food Biotechnology*, **6(4)**: 255–263. https://doi.org/10.22037/afb.v6i4.25906

Uthayasooriyan, M., Pathmanathan, S., Ravimannan, N. & Sathyaruban, S. 2016. Formulation of alternative culture media for bacterial and fungal growth. *Der Pharmacia Lettre*, **8(1)**: 444–449.

Valdez-Calderón, A., Barraza-Salas, M., Quezada-Cruz, M., Islas-Ponce, M.A., Angeles-Padilla, A.F., Carrillo-Ibarra, S., Rodríguez, M., Rojas-Avelizapa, N.G., Garrido-Hernández, A. & Rivas-Castillo, A.M. 2020. Production of polyhydroxybutyrate (PHB) by a novel *Klebsiella pneumoniae* strain using low-cost media from fruit peel residues. *Biomass Conversion and Biorefinery*, **12:** 1–14. https://doi.org/10.1007/s13399-020-01147-5

Vieira, I.M.M., Santos, B.L.P., Silva, L.S., Ramos,L.C., de Souza, R.R., Ruzene, D.S. & Silva, D.P. 2021. Potential of pineapple peel in the alternative composition of culture media for biosurfactant production. *Environmental Science and Pollution Research*, **28(48)**: 1–15. https://doi.org/10.1007/s11356-021-15393-1