ASIAN JOURNAL OF HEALTH AND APPLIED SCIENCES
2022, VOL. 1, NO. 2, 24–31
https://doi.org/10.53402/ajhas.v1i2.35

LIGHTHOUSE

OPEN ACCESS

Mung beans (*Phaseolus radiates* L.): Utilization as components of the growth medium *Bacillus subtilis*

Wasiyah Khusna Fadhilah[1*]
Heru Agung Saputra[2]
Nimisha Baby[3]

[1] Medical Laboratory Technology, Institut Kesehatan Rajawali, Indonesia
[2] Institute of BioPhysio Sensor Technology (IBST) and Department of Chemistry, Pusan National University, Busan, Republic of Korea
[3] Department of Chemistry, T.M. Jacob Memorial Government College Manimalakukunu, Kerala, India

ABSTRACT
Bacillus subtilis is a bacterial contaminant widely discovered in laboratories and used in educational needs. Synthetic media are relatively expensive, which makes it difficult for microbiology laboratories to meet large-scale bacterial growth media demands. This has encouraged researchers to find alternative media with cheaper and easily available prices, namely Mung Beans, which are known to contain protein nutrients that are good for bacterial growth, which is as much as 24%. The purpose of this study was to determine the potential of mung beans' alternative media as a medium for bacterial growth. This research is a descriptive study and the sample used is 1 tube of a pure isolate of *Bacillus subtilis*. The variable of this research is mung beans as an alternative medium for the growth of *Bacillus subtilis*. The results were obtained by observed macroscopically and microscopically, followed by a carbohydrate fermentation test and an IMViC test. Then showed that the colonies growing on the alternative medium of mung beans were in accordance with the characteristics of the colonies growing on Nutrient Agar medium (control), which indicated that these characteristics were *Bacillus subtilis*. Based on the research that has been done, it can be concluded that there is a qualitative growth of *Bacillus subtilis* in mung beans alternative media, which means that mung beans alternative media has the potential as a growth medium for *Bacillus subtilis*.

KEYWORDS
Mung beans; alternative medium; *Bacillus subtilis*

Received: June 9, 2022
Accepted: June 29, 2022
Published: June 30, 2022

Introduction

The presence of microorganisms can cause contamination, which has a significant impact on sterile spaces such as surgery rooms, labs, and other areas. *Bacillus subtilis*, a common contaminant microbe that can grow in a nutrient agar medium, is frequently detected in microbiology laboratories (Ghayoor, 2015).

Bacillus subtilis is a Gram-positive bacteria with endospores that function to protect the organism from extreme environmental conditions. *Bacillus subtilis* has a rod shape (thick or thin), double or single chains, and is motile by flagella. *B. subtilis* has proven to be relatively easy to manipulate genetically, and it has been frequently used as a model organism for laboratory study, particularly in the context of sporulation, which is a simple example of cellular differentiation. B. subtilis is commonly regarded as the Gram-positive equal of Escherichia coli, a well-researched Gram-negative bacteria, in terms of popularity as a laboratory model organism (Martinez, 2013).

Bacillus subtilis is not directly included as a pathogen in humans. However, *Bacillus subtilis* can contaminate food (such as dairy products, meat, rice, and pasta) because it provides good nutrition for growth but does not cause food to be toxic (Ryan and Ray 2004). In the laboratory, nutrients for bacterial growth are present in the medium. In addition to growth, the medium also functions for isolation, propagation, testing of physiological properties, and calculating the number of microorganisms (Waluyo, 2008).

The general medium that is usually used for bacterial growth in educational institutions and microbiological laboratories is the Nutrient Agar medium, a nutrient agar medium made in a ready-to-use form by certain factories and companies. Synthetic medium is widely used as a medium for bacterial growth because it is easy to manufacture. However, the relatively high price is an obstacle for several educational institutions and microbiology laboratories in learning facilities and research needs that use large quantities of medium-made materials. The use of alternative media will facilitate the learning process for students as well as the research process carried out in the microbiology laboratory because the materials used are easy to obtain and have low prices. Several researchers have succeeded in making microorganism growth medium from easily found natural resources such as cassava starch (Kwoseh, Darko, Adubofour, 2012), soybeans for the growth of Salmonella typhi and Staphylococcus aureus (Maharani, 2014), cassava,

CONTACT Wasiyah Khusna Fadhilah wasiyahkf02@gmail.com

white sweet potato, sweet potato. yellow sweet potato for the growth of *E.coli* and *Bacillus subtilis* bacteria (Ariyanti, 2016), jackfruit seeds and kluwih seeds for the growth of *Bacillus subtilis* (Lestari, 2016).

Mung beans (*Phaseolus Radiatus.* L) have a fairly high protein content, which is as much as 24%. Compared to other natural resources that have been studied, such as cassava, white sweet potato, and yellow sweet potato, mung beans have a higher nutritional content, especially protein content. In 100 g of mung bean flour, there is 4.5 g protein, 83.5 g carbohydrates, 1.0 g fat, 50.0 mg calcium, 100 mg phosphorus, and 1 mg iron. In the form of seeds, the protein content reaches 27.9%, fat 0.8%, carbohydrates 36.2%, starch 19.0%, and sugar 3.13% (Yusuf, 2014). Based on this description, it is suspected that mung beans can be used as an alternative medium for bacterial growth because they contain good nutrients for bacterial growth.

Based on the above, the researcher proposes to research and develop a Nutrient Agar (instant medium) alternative medium for gram-positive bacteria, specifically *Bacillus subtilis*, utilizing Mung Bean Seeds (*Phaseolus Radiates.* L) as a growth medium for *Bacillus subtilis.* Mung bean medium (*Phaseolus Radiates.* L) has the advantage of being a cost-effective, easy-to-obtain alternative medium for bacterial growth that supplies a source of nutrients for bacterial growth.

Methods

Mung beans (*Phaseolus radiates. L*)

Mung beans are the third legume crop that is widely cultivated after soybeans and peanuts. When viewed from the suitability of the climate and the condition of the land owned, Indonesia is one of the countries that have the opportunity to export mung beans (Purwono and Hartono, 2005). Mung bean fruit is an elongated round pod that ranges in size from 6 to 15 cm. Inside each fruit, there are 5-10 seeds of mung beans. There are seeds that are shiny and some that are dull, depending on the type. Mung bean seeds are round or oval and generally mung, but some are yellow, brown, or black speckled. The two most well-known types of mung beans are the golden gram and the mung gram. In botanical terms, golden gram is a mung bean that is golden in colour and is known as Phaseolus aureus. While *Phaseolus radiates* refers to mung gram (Astawan, 2009), mung peas are short, upright-branched plants. Parts of the mung bean plant include roots, stems, leaves, flowers, fruit, and seeds.

Bacterial Growth Medium

Solid media is typically used in cultivating microorganisms and identifying the bacteria. Nutrient Agar (NA) and Blood Agar Plate (BAP) are two types of media that can be used to culture bacteria. Nutrient Agar is a medium in solid form, which is a combination of natural ingredients and chemical compounds. NA is made from a mixture of meat extract and peptone using agar as a solidifier. In this case, it should be used as a compactor because it is easy to freeze and contains carbohydrates in the form of galactan so that it is not easily broken down by microorganisms. In this case, beef extract and peptone are used as basic ingredients because they are a source of protein, nitrogen, vitamins, and carbohydrates, which are needed by microorganisms to grow and develop. This medium, Nutrient Agar, is a medium that is light brown in colour and has a dense consistency. This medium is of synthetic origin and is used as a medium for growing bacteria. This nutrient medium usually contains: 0.5% peptone, 0.3% beef extract or yeast extract, 1.5% agar, and 0.5% NaCl (Harry, 2012).

A blood Agar Plate is one type of solid medium used for the identification of microorganisms. A blood Agar Plate is a selective and differential medium for microbes and is also a selective medium for the isolation and identification of Gram-positive bacteria. Differential media is a medium that is added with certain chemicals so that a microorganism forms a growth to classify a group of types of bacteria. Blood Agar Plate distinguishes hemolytic and non-hemolytic bacteria based on their ability to lyse red blood cells. The composition of the Blood Agar Plate contains 15 grams of tryptone, 5 grams of a soy peptone, 5 grams of sodium chloride, 10 grams of lithium chloride, 3.8 grams of magnesium sulfate, and 15 grams of agar (Harry, 2012).

Bacillus subtilis

Bacillus subtilis cells are bacilli-shaped; they are thick and thin. Usually in chain form or separate. Some are motile and some are non-motile. All form endospores which are round and oval in shape. *Bacillus subtilis* is a type of thermophilic bacteria that can grow in the temperature range of 45 ˚C–55 ˚C and has an optimum temperature range of 60 ˚C–80 ˚C (Graumann, 2007).

Bacillus subtilis is one type of bacteria that is widely used in observing the morphology of a bacterium. *Bacillus subtilis* belongs to the genus Bacillus. *Bacillus subtilis* has the ability to form protective endospores that give the bacteria the ability to tolerate extreme conditions. Unlike other species, historically, *Bacillus subtilis* is classified as an obligate anaerobe, although current research is not correct. *Bacillus subtilis* is not considered a pathogen even though it contaminates food and rarely causes food poisoning. The spores can withstand the high heat often used in food and are responsible for spoiling bread. *Bacillus subtilis* showed a spherical colony shape on the Nutrient Agar medium. The colonies are generally white to yellowish or white in colour. The edges of the colonies vary but are generally uneven. The surface is rough and not slimy. Some even tend to be dry to powdery. The colonies are large and not shiny. The shape and size of the colony vary greatly depending on the species. In addition, each type also shows different abilities and resistance in dealing with environmental conditions, for example, resistance to heat, acid, salt content, and so on (Madigan, 2005).

Methods

This study is a descriptive study that aims to determine whether mung beans (*Phaseolus Radiates.* L) can be used as an alternative medium for the growth of *Bacillus subtilis*.

Sample

The population in this study was a pure isolate of *Bacillus subtilis*, and the sample was *Bacillus subtilis*. The sample size used was 1 tube of a pure isolate of *Bacillus subtilis*. The variable of this research is mung beans as an alternative medium for the growth of *Bacillus subtilis*.

Data collection

The sampling technique used in this research is purposive sampling. The isolates must be completely pure Bacillus subtilis with the characteristics of being obtained from a single colony and not mixed with other colonies (free of contamination). The data used in this research are primary data. The primary data was obtained through a laboratory examination, which consisted of isolating *Bacillus subtilis* on a mung beans alternative medium, observing its growth, and comparing it to Nutrient Agar (NA) medium as a control medium.

The first step in this research is a determination test that aims to get a specific species on target. Furthermore, the pre-analytic stage is carried out, which includes the preparation of tools and materials that will be used in the study. Furthermore, a preliminary test is carried out, which aims to find out the obstacles and also serves as a reference for researchers that this research can really be done. After that, the initial testing procedure was carried out, namely a preliminary test on a pure strain of *Bacillus subtilis*. Then the testing procedure was carried out, with the first step is identifying and culturing *Bacillus subtilis* bacteria on Nutrient Agar medium and mung beans alternative medium. After obtaining the results, the data was collected and analyzed to draw conclusions and then reported.

Data analysis

The data taken is primary data by observing the alternative medium of mung beans and whether it is overgrown by *Bacillus subtilis*. If there is growth observed macroscopic and microscopic, followed by a carbohydrate fermentation test and an IMViC test. Then compared the isolates grown in mung bean medium with the positive control isolates of pure *Bacillus subtilis* nutrient agar medium. The data are presented in the form of tables and narratives according to the descriptive research.

Results and Discussion

Identification of Bacillus subtilis

Identification is obtained by macroscopic and microscopic examinations. Macroscopic examination of material in the form of pure isolates measured on a selective medium, Blood Agar Plate (BAP).

Table 1. Characteristics of *Bacillus subtilis* colonies grown on BAP medium incubated at 37º C, and 24 hours of culture

Observed aspects	Observation result
Type	Round
Colour	Whitish
Trait	Not Slimy
Elevation	Flat
Edge	Flat
Size	3-4 mm

Figure 1. Colonies of *Bacillus subtilis* on Blood agar plate, Incubated at 37º C for 24 hours

Microscopic examination of material in the form of pure isolates was prepared on a slide and stained with Gram stain.

Table 2. Characteristics of Pure Cell Isolates *Bacillus subtilis* Microscopic 1000x magnification Gram stain

Observed aspects	Observation result
Type	Rod
Colour	Purple
Structure	Single and Chains
Trait	Gram-Positive

IMViC test

The IMViC test, which included the Indole Test, Methyl Red and Voges-Proskauer (MR-VP), and Citrate Test, was used to distinguish enteric microorganisms. The IMViC test results are shown in Table 3.

Table 3. IMViC test results on Medium Blood agar plate (BAP) and Mung Bean Agar

Test	Observation result
Indol	Negative
Methyl Red	Positive
Voges-Proskauer (VP)	Negative
Simmon Citrate	Positive
Carbohydrate Fermentation	Positive

Preliminary test

Preliminary testing is carried out first to avoid recurrent study and research failures. The findings of *Bacillus subtilis'* macroscopic test on Nutrient Agar Medium are shown in table 4.

Table 4. Characteristics of Colonies of *Bacillus subtilis* on Nutrient Agar Medium (Preliminary Test)

Aspects Observed	Observation result
Type	Round
Colour	White
Edge	Flat
Elevation	Flat

Observation data on macroscopic test growth of *Bacillus subtilis* colonies on mung beans alternative medium are shown in table 5.

Table 5. Characteristics of Colonies of *Bacillus subtilis* on Mung Beans Alternative Medium

Aspects Observed	Observation result
Type	Round
Color	White
Edge	Flat
Elevation	Flat
Measure	2-3 mm

The results obtained showed that the colonies growing on the alternative medium of mung beans were in accordance with the characteristics of the colonies growing on the Nutrient Agar medium (control). This indicated that these characteristics were *Bacillus subtilis* are shown in Figure 3. below:

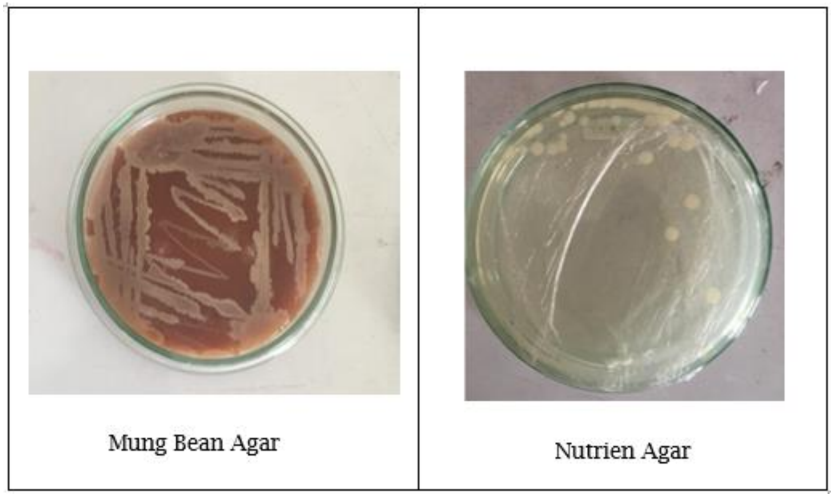

Figure 3. Preliminary Test Results of Colony Growth of *Bacillus subtilis* On Mung Beans Alternative Medium and On Nutrient Agar Medium.

Results of observations on the utilization of mung beans as a component of the growth medium for Bacillus subtilis

This test was carried out using the streak quadrant method by isolating *Bacillus subtilis* bacteria in an alternative medium of mung beans and Nutrient Agar medium as a control. Then incubated at 37 ˚C for 24 hours. The result of the research are reported in table 6. below:

Table 5. Growth Results of *Bacillus subtilis* On Nutrient Agar Medium And Alternative Medium Of Mung Beans Agar

Bacteria	The presence or absence of bacterial growth on the control medium (+) NA	The presence or absence of bacterial growth on mung beans alternative medium			Description
		Repetition 1	Repetition 2	Repetition 3	
Bacillus subtilis	√	√	√	√	Grow

The growth results of *Bacillus subtilis* on Nutrient Agar medium and Mung Beans Alternative medium were found that there was growth in the alternative medium of mung beans repetition 1, 2, and 3 and Nutrient Agar medium as a positive control.

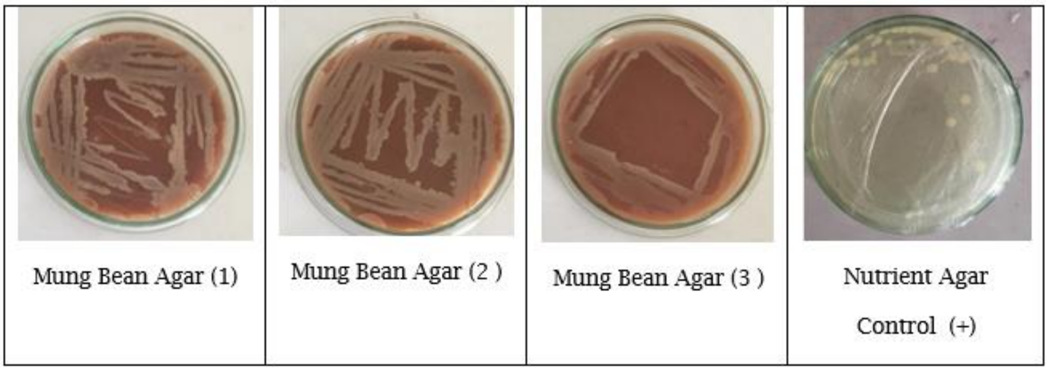

Figure 4. Growth Results of *Bacillus subtilis* On Nutrient Agar Medium And Alternative Medium Of Mung Beans Agar.

Table 6. Characteristics of Colonies of *Bacillus subtilis* on Nutrient Agar Medium

Aspects Observed	Observation result
Type	Round
Colour	White
Edge	Flat
Elevation	Flat
Measure	2-3 mm

Data from research on the growth of *Bacillus subtilis* colonies on an alternative Mung Beans medium:

Table 7. Characteristics of Colonies of *Bacillus subtilis* on Mung Beans Agar Medium

Aspects Observed	Observation result
Type	Round
Colour	White
Edge	Flat
Elevation	Flat
Measure	2-3 mm

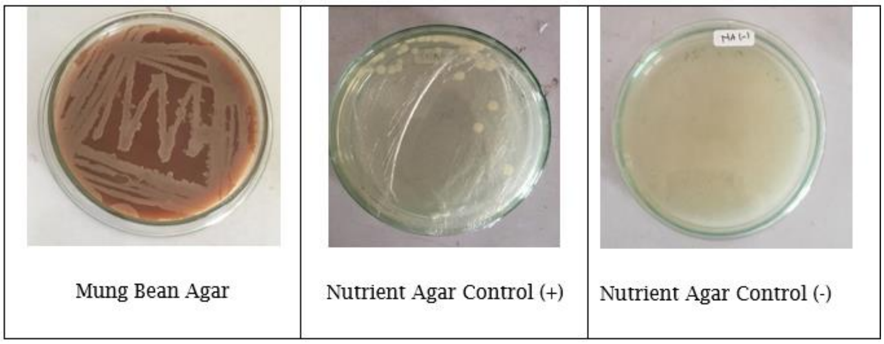

Figure 5. Growth Results of *Bacillus subtilis* On Nutrient Agar Medium And Alternative Medium Of Mung Beans Agar

Colonies growing on mung beans alternative media were microscope examination, IMViC test and Carbohydrate Fermentation Test:

Table 7. Characteristics of Colonies of *Bacillus subtilis* on Mung Beans Agar Medium

Aspects Observed	Observation result
Type	Round
Colour	White
Structure	Single
Trait	Gram-Positive

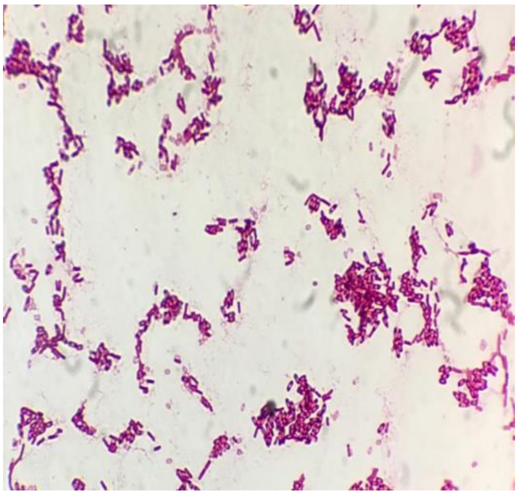

Figure 5. Microscopic Observation Results of *Bacillus subtilis* at 1000x magnification On Alternative Medium Of Mung Beans Agar

Table 8. Results of microscopic examination of IMViC Test and Carbohydrate Fermentation Test of colony growth on the alternative medium of Mung beans

Biochemistry Test	Observation result
Indole	Negative
MR	Positive
VP	Negative
SC	Positive
Glucose	Positive
Lactose	Negative
Sucrose	Positive

Discussion

The initial stage of the research was the identification of *Bacillus subtilis* by means of macroscopic and microscopic observations, followed by a carbohydrate fermentation test and an IMViC test. The results of the identification of *Bacillus subtilis* are in accordance with the references described by Cappuccino & Sherman (2008), namely microscopically in the form of rods, Gram-positive with a single bacterial arrangement as well as chains, and purple cells. On Blood agar plate medium, *Bacillus subtilis* produced spherical colonies, flat elevations, and pale white in colour. From these microscopic and macroscopic observations, it was known that this pure culture was indeed *Bacillus subtilis*, but to confirm that the bacteria was indeed *Bacillus subtilis*, this test was continued to the biochemical test stage, namely the carbohydrate fermentation test and the IMViC Test. A biochemical and confectionery medium is a medium used to determine the characteristics of microorganisms by inoculation on certain mediums. Conventionally, this medium is used to identify the characteristic biochemical properties of certain microorganisms (Swarjana, 2012).

The results of the carbohydrate fermentation test are in accordance with the references described by Cappuccino & Sherman, (2008), namely *Bacillus subtilis* which produces positive results for glucose fermentation, negative for lactose fermentation, and positive for sucrose fermentation. In the IMViC test, negative results were obtained on the indole test which was indicated by the absence of a red ring on the surface of the broth, this result indicated that Bacillus subtilis could not form indole from the degradation of the amino acid tryptophan. Furthermore, in the MR-VP or Methyl Red and Voges Proskauer tests, positive results on the MR test were indicated by a colour change to red which indicates that glucose can be converted into acids such as lactic acid, acetic acid, or formic acid, and negative in the VP test is marked with no colour change indicating that glucose cannot be converted into acetyl methyl carbinol. In the Simmon's Citrate test the results obtained were a colour change to bright blue which indicated that *Bacillus subtilis* could convert citrate to oxaloacetate. From the identification results, it was found that the pure culture obtained was the *Bacillus subtilis*.

The results of the data analysis showed that *Bacillus subtilis* can grow on an alternative medium of mung beans and nutrient agar media. *Bacillus subtilis* colonies grew on mung beans medium. The colonies were white, circular in form, with flat borders and flat elevation, the same as the colonies growing on NA media. Based on morphological research from Jawetz, Melnick, and Adelberg (2005), particularly the results of colonies growing on an alternate medium of mung beans and nutrients, it is thought to be ideal. This might be because of the nutritious value of the mung bean medium.

The factor that causes bacteria to grow is due to nutrients such as protein, vitamins, and minerals. Mung beans contain sufficient nutrients to make the bacterial growth process optimal so that bacteria can grow well. The nutritional content contained in mung beans, according to Hartono and Purwono (2005), in 100 grams contains 22.00 g protein, 62.90 g carbohydrates, 1.20 g total fat, and many vitamins and minerals that are sufficient.

The growth of microorganisms in an artificial medium is influenced by several physical and chemical factors. Physical factors include pH and temperature, while chemical factors include nutrients needed by microbes, for example, from protein sources. Proteins in bacterial growth function as the main constituents of bacterial cell structures such as cell walls, cell membranes, flagella, cytoplasm, and pili. Carbohydrates are needed by bacteria as the main substrate for bacterial metabolism. Vitamins function to form substances that activate enzymes that cause chemical changes. In addition, bacteria need minerals for metabolic functions and growth. Mineral elements also function to regulate the osmotic pressure of H+ ion levels (acidity, pH). Water is the main component of microbial cells and the medium. The function of water is as a source of oxygen for cell organic matter in respiration. In addition, water functions as a solvent and a means of transport in metabolism.

The results of microscopic observations on the alternative medium of mung beans agar and NA medium show differences in the composition and relative amounts of the gram staining results, which indicate that *Bacillus subtilis* in the alternative medium of mung beans obtained relatively higher amounts and the composition is only a single, while in NA medium the arrangement is in the form of a single and also a chain. This is presumably because there are differences in protein contained in the alternative medium of mung beans agar and NA medium. The components in the alternative medium of mung bean agar have been cultivated to have a protein-rich content, but there are still differences, namely in the form (composition) of protein between the alternative medium and the control medium. In the control medium, the protein contained was in a simpler form or structure so that the bacteria digested it more easily. Thus, the findings of this study indicate that mung beans can be utilized as an alternative medium for the development of *Bacillus subtilis*.

Conclusion

The results of the study "Utilization of Mung Bean Seeds (*Phaseolus radiates* L.) as Components of The Growth Medium *Bacillus subtilis"* showed that the growth of *Bacillus subtilis* in the alternative medium of mung beans is such that it has the potential as a growth medium.

Acknowledgements

The authors are grateful to the all parties in Medical Laboratory Technology, Institut Kesehatan Rajawali, Bandung, Indonesia; Institute of BioPhysio Sensor Technology (IBST) and Department of Chemistry, Pusan National University, Busan, Republic of Korea; And Department of chemistry, T.M.Jacob Memorial Government College Manimalakukunu, Kerala, India.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

Ariyanti, Widya. (2016). *Pertumbuhan Bakteri E.coli dan Bacillus subtilis Pada Media Singkong, Ubi Jalar Putih, dan Ubi Jalar Kuning Sebagai Subtitusi Media NA*. Surakarta: Universitas Muhammadiyah.
Astawan, MS. (2009). *Sehat dengan hidangan kacang dan biji–bijian*. Jakarta: Penebar Swadaya.
Capuccino, J. G. and Sherman, N. (2008) *Microbiology a Laboratory Manual*. California: The Benjamin/Cummings Publish.
Ghayoor, M., Qadoos, A., Bahadar, S., Hayat, A., Daud, M., Hassan, A., Ali, F., Zeb, A., Rahman, K.U., Wahab, A., & Fatima, Z. (2015). Isolation and Identification of Common Contaminants Bacteria from Working Area in Microbiology Laboratory. *Journal of Bio–Molecular Sciences*, 3(2), 74-78.
Harry. (2012). *Komposisi Nutrient Agar dan Nutrient Broth dan Kegunaannya*. Gramedia: Samarinda.
Hartono, R., and Purwono, M.S. (2005). *Kacang hijau*. Bogor: Penebar Swadaya.
Jawetz, M. dan Adelberg. (2005). *Mikrobiologi Kedokteran (Medical Microbiology)*. Jakarta: Salemba Medika.
Kwoseh, C. K., Darko, M. A., and Adubofour, K. (2012). Cassava Starch-Agar Blend as Alternative gelling Agent for Mycological Culture Media. Bots. *J. Agric. Appl. Sci*., *8*(1), 8-15.
Lestari, D. P. Y. (2016). *Pertumbuhan bakteri Bacillus subtilis pada media biji nangka dan biji kluwih sebagai substitusi media NA (Nutrient Agar)*. Surakarta: Universitas Muhammadiyah.
Madigan, M. (2005). *Brock Biology of Microorganism*. Englewood Cliff: Prentice-Hall.
Martinez R. M. (2013). *Bacillus subtilis. Brenner's Encyclopedia of Genetics (Second Edition)*. University of Washington, Seattle, WA, USA: Academic Press.
Ryan K.J., Ray, C. G. (2004). *Sherris Medical Microbiology*. Ed ke-4. New York (US): McGraw Hill Book Company Inc.
Swarjana, I. K. (2012). *Metodologi penelitian kesehatan*. Yogyakarta: CV Andi Offset.
Yusuf. (2014). Pemanfaatan Kacang Hijau sebagai Pangan Fungsional Mendukung Diversifikasi Pangan di Nusa Tenggara Timur. *Prosiding Seminar Hasil Penelitian Tanaman Aneka Kacang dan Umbi*, 741-746.
Waluyo L. (2008). *Teknik dan metode dasar dalam mikrobiologi*. Malang: Penerbit Universitas Muhammadiyah Malang.