Making *Bacillus cereus* Bacterial Growth Media from Soybeans (*Glycine max* (L.) Merril)

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Abstract. This study develops a selective medium for the growth of *Bacillus cereus* from soybeans, which can be directly applied in the laboratory to replace media made from meat extract. This study considers the economic aspect by making inexpensive microbiological media, as well as testing the halal aspect so that it can be applied to halal-certified industries. The sizes of soybean powder used were 44 mesh, 60 mesh, and 100 mesh. While the concentration of soybeans in the media 1, 2, 3, and 4 g/200 ml. The media is made by mixing soybean powder with the addition of mannitol, sodium chloride, phenol red, agar, and aquades. Media Mannitol Egg Yolk Polymyxin Agar is used to isolate *Bacillus cereus* bacteria, which is very widely used in industry and microbiology laboratories. Soybeans have a fairly cheap price compared to meat extract. Soybeans can be used as an alternative raw material for the manufacture of bacterial growth media. Soybeans are a source of high protein for the growth of *Bacillus cereus* bacteria, which can replace the presence of meat extract in standard media. The results showed that the optimal number of bacterial colonies of *Bacillus cereus* occurred at a concentration of 4 g/200 ml soybean powder and 100 mesh size of soybean powder with a maximum number of colonies of 8.67 CFU/ml. While the optimal bacterial colony size of *Bacillus cereus* occurred at a concentration of soy powder 4 g/200 ml, and soybean powder size 100 mesh with a maximum colony size of 15.74 mm. Soybeans can be used for the manufacture of halal and cheap *Bacillus cereus* bacterial growth media with quality equivalent to media made from meat extract.

Keywords: Bacillus cereus · Soybean · Bacterial Media

1 Introduction

Soybeans have an important role in meeting domestic food needs. With adequate nutritional content as food, soybeans can also be used for non-food ingredients such as protein sources for microbiology laboratory analysis. In 2018 it is estimated that soybean harvest will increase by 91.22% to 680.37 thousand hectares from the previous year of 355.79 thousand hectares. Soybean production in Indonesia in the 2014–2018 period fluctuated and tended to increase with an average growth of 8.79% per year [1]. The soybean plant used in this study was white soybean from the Bringin area, Semarang Regency with

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the species name *Glycine max* (L.) Merril. The classification of soybean plants (*Glycine max* (L.) Merril) is as follows.

Kingdom:	*Plantae*
Division:	*Spermatophyta*
Subdivision:	*Angiosperms*
Class:	*Dicotyledoneae*
Order:	*Rosales*
Family:	*Laguminoseae*
Species:	*Glycine max (L.) Merril*

Soybean is an annual plant in the form of low shrubs, erect plants, dense leaves, and various morphologies. This soybean plant height ranges from 10–200 cm, and can be branched a little or a lot. Cultivars with broad leaves can give higher yields because they can absorb more sunlight when compared to narrow leaves [2].

The structure of the soybean plant body consists of 2 main types of organs (organs), namely vegetative organs and generative organs. Vegetative organs include roots, stems, and leaves that function as a means of taking, transporting, processing, distributing, and storing food so that they are called nutrient tools (*organum nutritivum*). Meanwhile, generative organs include flowers, fruits, and seeds that function as reproductive organs [3].

In this study, the protein content of soybean powder was determined using the Kjeldahl method. The Kjeldahl method is widely used throughout the world, including in industry. The Kjeldahl method is still the standard method used for determination of protein content. Its universal nature, high precision and good reproducibility make this method widely used for protein assays. Kjeldahl method determines total protein content by calculating the element nitrogen in the sample. The Kjeldahl method goes through three stages, namely the process of destruction, distillation, and titration. The Kjeldahl method is a method that is quite accurate and specific enough to determine the amount of protein by determining the nitrogen content in soybeans. The Kjeldahl method has drawbacks that purines, pyrimidines, vitamins, large amino acids, and creatine are also analyzed and measured as nitrogen. However, this method is still used and is considered quite accurate to be used as a determinant of protein content [3].

With adequate nutritional content as food, soybeans can be used for non-food ingredients such as protein sources for microbiology laboratory analysis [4]. Soybeans have an important role as a source of functional food in regarding the nutritional content of soybean seeds [5]. Based on dry weight basis, soybean contains about 40% protein, 20% oil, 35% carbohydrates (sucrose, stachyose, raffinose, etc.) and insoluble carbohydrates (dietary fiber), and 5% ash [6]. Besides being used as a protein source, since the early 2000s, soybean oil has also been used as a bioenergy material in the form of diesel oil [7]. Soybeans are a complete protein, cheap, and are one of the foodstuffs that contain essential and non-essential amino acids, carbohydrates, fats, vitamins, and minerals. Soybeans contain minerals K, P, Ca, Mg, and Fe, as well as other useful nutritional components, such as isoflavones that function to prevent various diseases [8].

Amino acids are related to each other in a unique and sequential order called the primary structure of a protein. Polypeptides can be folded or rolled, which causes the

emergence of a secondary structure [9]. Protein from the stomach contents of fish carp can be used as a nutrient in the bacterial growth medium and as a substitute for using commercial peptone [10]. Proteins that contain ingredients other than amino acids, such as vitamin derivatives, fats, and carbohydrates, are called complex proteins [11]. Proteins that contain ingredients other than amino acids, such as vitamin derivatives, fats, and carbohydrates, are called complex proteins [3]. Growth factors are organic molecules that are important for growth but cannot be synthesized by microbes themselves, such as vitamins and amino acids.

Bacillus cereus bacteria belong to the group of spore-forming facultative anaerobic bacteria. *Bacillus cereus* is a gram-positive bacterium commonly found in soil. In general, *Bacillus cereus* colonies that were not detected by Mannitol Egg Yolk Polymyxin media were caused by the presence of other competitive bacteria. There was a significant difference between mannitol egg yolk polymyxin and bacara for rice, infant formula, potato, and milk products. Bacara can detect *Bacillus cereus* at lower concentrations than mannitol egg yolk polymyxin. For macaroni and cheese products, there was no significant difference between mannitol egg yolk polymyxin and bacara in detecting the presence of *Bacillus cereus* [12].

Soybeans can be used as a source of microbial protein by the research of regarding the effectiveness of the concentration of soy powder as a substitute for peptone in *Candida albicans* growth media [13]. Mannitol egg yolk polymyxin agar generally contains nutrients or protein derived from meat extract and bacto peptone. Beef extract and peptone are used as basic ingredients because they are a source of protein and nitrogen that are needed by microorganisms. Research on using soybeans for the manufacture of mannitol egg yolk polymyxin agar media is also intended to ensure the halalness of mannitol egg yolk polymyxin agar media, due to the substitution of vegetable protein sources [14]. The novelty aspect of this research is to make selective media for microbiological testing from soybeans that can be directly applied in the laboratory to replace media made from meat extract. This study used *Bacillus cereus* bacteria as a tester for the performance of soybean media, while previous studies used *Staphylococcus* and *Salmonella Typhi* bacteria. This study examines the economic aspect of making cheaper microbiological media, and examines the halal aspect of the media that is made so that it can be applied to halal-certified industries.

2 Experimental

This study used a completely randomized design (CRD) with two factors and 3 repetitions. The first factor is the weight of soybean powder consisting of five treatment levels. The second factor is the size of the soybean powder, which consists of three treatment levels. The treatment in this study was a combination of factors from all levels of treatment. In this study there were 3×5 combinations or 15 combinations.

Factor I is the concentration of soybeans consisting of 5 levels of treatment, namely:

K0 = Control 0 g/200 ml.
K1 = Soybean powder weighing 1 g/200 ml
K2 = Soybean powder weighing 2 g/200 ml

Table 1. Combination of Soybean Weight Treatment and Soybean Powder Size

Soybeans Powder Size (U)	Concentration (K)				
	K0	K1	K2	K3	K4
U1	U1 K0	U1 K1	U1 K2	U1 K3	U1 K4
	U1 K0	U1 K1	U1 K2	U1 K3	U1 K4
	U1 K0	U1 K1	U1 K2	U1 K3	U1 K4
U2	U2 K0	U2 K1	U2 K2	U2 K3	U2 K4
	U2 K0	U2 K1	U2 K2	U2 K3	U2 K4
	U2 K0	U2 K1	U2 K2	U2 K3	U2 K4
U3	U3 K0	U3 K1	U3 K2	U3 K3	U3 K4
	U3 K0	U3 K1	U3 K2	U3 K3	U3 K4
	U3 K0	U3 K1	U3 K2	U3 K3	U3 K4

K3 = Soybean powder weighing 3 g/200 ml
K4 = Soybean powder weighing 4 g/200 ml

Factor II is the size of soybean powder consisting of 3 levels of treatment, namely (Table 1):

U1 = 44 mesh
U2 = 60 mesh
U3 = 100 mesh

The variables in this study consisted of independent variables and dependent variables.

1. The independent variable consisted of soy powder concentration (g/l), namely K0 = 0 g/200 ml (control), K1 = 1 g/200 ml, K2 = 2 g/200 ml, K3 = 3 g/200 ml, and K4 = 4 g/200 ml. While the size of soybean powder is U1 = 44 mesh, U2 = 60 mesh, and U3 = 100 mesh.
2. The dependent variables consisted of soybean varieties, growth of *Bacillus cereus* colonies on media containing soybeans and meat extract, incubation temperature, sterilization temperature and time, and soy protein content.

2.1 Materials

2.1.1 Main Materials

The main ingredient used in this study was soybeans (*Glycine max* (L.) Merril). The ingredients are dry soybeans that have been sorted to get the best soybeans.

2.1.2 Auxiliary Materials

There are 14 auxiliary materials used in this study, namely aquadest, *Bacillus cereus* selective supplement SR0230, bacto agar, boric acid, egg yolk emulsion SR0047, H_2SO4, HCl, hydrogen peroxide, mannitol, mannitol egg yolk polymyxin agar CM0929, NaCl, NaOH, phenol red, *Bacillus cereus* strains ATCC 11778.

2.2 Tools

The tools used in this research are autoclave (Hirayama), oven (UFB 500), destruction unit FOSS tecator, scrubber FOSS, kjeltec 2100 distilling unit (Parts List 1000 9405/Rev. 1.2), waterbath (Memmert WB 436-D Funke Gerber), petri dish, pH meter (Schott-CG842), hot plate stirrer (Thermo SP131320–33), electronic balance (Mettler Toledo/PB4002-S), incubator (Memmert/INB-500), laminar air flow (Esco AVC-4AI), 44, 60, and 100 mesh sieve.

2.3 Preparation of Media

2.3.1 Tool Sterilization

Analytical equipment such as petri dishes, erlenmeyer, micro pipette, spreader, finntip, measuring cups, and test tubes were wrapped in heat-resistant plastic or aluminum foil and then sterilized in an autoclave at 121 °C for 20 min. Next, the equipment was dried in an oven at 160 °C for 2 h (ISO 7932:2004).

2.3.2 Selection of Soybean Seeds

Selection of soybean seeds aims to obtain soybeans with the best possible quality that can be obtained easily in the area around the study. Soybeans selected must be ripe or ready to harvest, that is yellow or dark yellow. Ripe soybeans have a high protein content.

2.3.3 Soybean Powder Making

The dried soybean seeds were ground until smooth and then filtered using sieves with sizes of 44, 60, and 100 mesh. Soybean powder obtained is then stored in a dry and closed container.

2.3.4 Soybean Powder Protein Analysis

Soybean powder obtained was determined for protein content using the Kjeldahl method to obtain % dissolved nitrogen. To obtain the soy protein content multiplied by a correction factor of 5.75 (Fig. 1).

$$\%\ \text{Protein} = \%\ \text{Nitrogen} \times 5.75$$

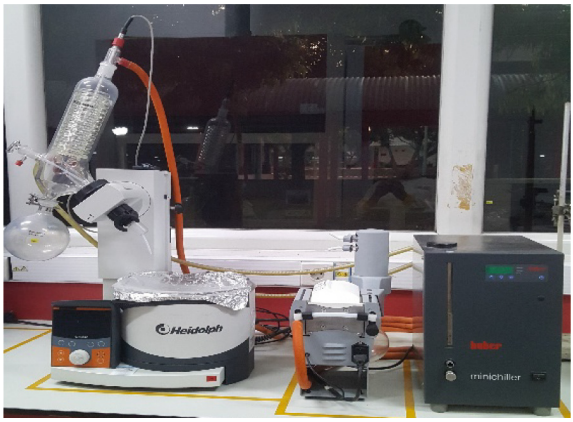

Fig. 1. Kjeltec 2100 Distilling Unit

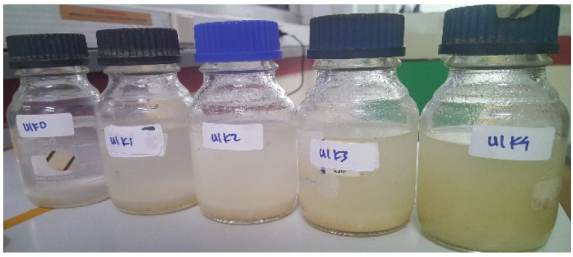

Fig. 2. Soybean MYP Media

2.3.5 Making Mannitol Egg Yolk Polymyxin Media Made from Soybeans

Mannitol egg yolk polymyxin media made from soybeans is made by dissolving soybean powder with additional ingredients of mannitol, NaCl, phenol red, agar, and aquades. Egg yolk emulsion and *Bacillus cereus* selective supplement were added after the media was sterilized by autoclave. The media that has cooled slightly is poured into the petri dish as much as ±15 ml until the media becomes solid, and dry (Fig. 2).

2.3.6 Growth Test of Bacillus Cereus on Mannitol Egg Yolk Polymyxin Media Made from Soybean Powder and Standard Mannitol Egg Yolk Polymyxin Media

Comparing the morphology of *Bacillus cereus* bacteria growing on soybean media and standard media made from meat extract. The number of bacterial colonies growing on soybean media and standards were counted and their diameter measured, besides that the color and shape of the colonies were also observed. Bacterial growth test of *Bacillus cereus* was carried out using the spread plate method where the sample was distributed on a petri dish containing solid media. Furthermore, the petri dish containing the sample was incubated for 48 h at a temperature of 300C (ISO 7932:2004). After the incubation

period was completed, the number of colonies was counted and the diameter of the presumptive colonies of *Bacillus cereus* was measured in each petri. Colony character is wide, pink (ISO 7932:2004). Identify bacterial colonies including their number, shape, size, and color. The calculated petri has a lot of colonies less than 150 (colonies are calculated from 1 ml of inoculum, so if 1 ml is distributed to 3 petries, the number of colonies/ml is the total number of colonies from the three petries). The character of the colony is wide, pink in color, this indicates that no mannitol fermentation has occurred and is usually surrounded by a precipitation zone indicating the presence of lecithinase production (ISO 7932:2004).

2.4 Formulation of the Problem

The formulation of the problem in this study is the effect of the concentration of soy bean powder in mannitol egg yolk polymyxin agar media on the growth of *Bacillus cereus* bacteria, the comparison of the growth of *Bacillus cereus* bacteria on mannitol egg yolk polymyxin agar media and soybeans and mannitol egg yolk polymyxin agar media made from meat extract, the next is the economic aspect and the halalness of the mannitol egg yolk polymyxin agar media product produced and its application in the food industry.

2.5 Aim

The purpose of this study was to determine the concentration and size of soybean powder, to obtain optimal growth of *Bacillus cereus* bacteria, to study the performance of mannitol egg yolk polymyxin agar media from soybeans.

2.6 Utility

This research is expected to be a reference to determine the optimal concentration and size of soybean powder in the manufacture of growth media for *Bacillus cereus* bacteria, increase the economic value of soybeans, and make cheap and halal growth media.

3 Result and Discussion

3.1 Analysis

Soy protein analysis using the Kjeldahl method obtained an average protein content of 35.95%. Under research conducted by Meng et al. (2019) the protein content in soybeans based on dry weight basis is 40%. The experimental results (Fig. 3) show that *Bacillus cereus* bacteria can grow on media made from soybeans. Soybean powder concentration and soybean powder size affect the number of bacterial colonies of *Bacillus cereus.* The highest number of colonies occurred at 100 mesh powder size and soybean concentration of 4 g/200 ml with an average colony number of 8.6667 CFU/ml. While the lowest number of bacterial colonies occurred at 44 mesh soybean powder size and soybean concentration of 1 g/200 ml, the growth of *Bacillus cereus* bacteria on average was 1 CFU/ml.

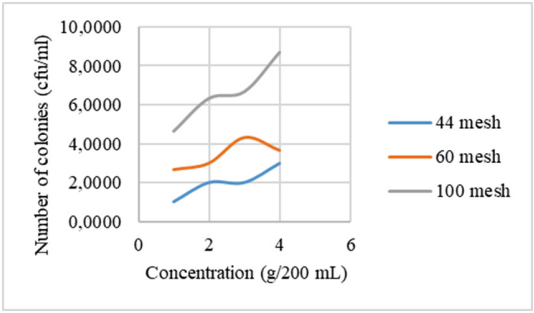

Fig. 3. Effect of Concentration and Size of Soybean Powder on the Number of Bacterial Colonies of *Bacillus cereus*

From this research, it is known that the concentration of soybean powder and the size of soybean powder affect the diameter of bacterial colonies. The largest colony diameter occurred at 100 mesh powder size and soybean concentration 4 g/200 ml with a colony diameter of 15.7350 mm. While the smallest colony diameter occurred at the size of 44 mesh soybean powder and soybean concentration of 1 g/200 ml with a colony diameter of 5,7667 mm. This happens because the higher the concentration of soy powder, the greater the amount of nutrients contained in the growth medium, so that the adequacy of nutrients for bacterial growth is more fulfilled and allows them to grow larger. These results are under Siti Danela's research (2019) which grew *Pseudomonas aeruginosa* bacteria using alternative media from soybean seeds. In this study, it was shown that the higher the concentration of soybeans, the better for the growth of *Pseudomonas aeruginosa* bacteria, as indicated by the larger size of the bacteria.

In Fig. 4 it can be seen that the diameter of the bacterial colony of *Bacillus cereus* increased from 44 mesh to 100 mesh. At the size of 100 mesh soybean powder, there was a very good growth of colony diameter and showed a pattern that continued to increase with increasing concentration. The smaller the size of the soybean powder, the higher the solubility, making it easier for bacteria to digest, this allows the colonies to grow larger. At 44 mesh and 60 mesh size of soybean powder, the growth of bacterial colony diameter was not good with an up and down and stagnant pattern. This happens because the size of the 44 mesh and 60 mesh powder is too large so that the solubility is low. With low solubility, soybean powder is not evenly distributed in the media and makes it difficult for bacteria to digest.

The comparison test between soybean-based media and standard media made from meat extract was carried out using a creamer sample. Creamer is a milk substitute product that is often used as a mixture in coffee and tea drinks to give the effect of taste and aroma. The creamer used in this study was in the form of a powder containing fat, protein, and glucose. Soybean mannitol egg yolk polymyxin agar can grow *Bacillus cereus* bacteria, which is present in each sample of creamer products analyzed, as well as for commercial in mannitol egg yolk polymyxin agar media standard media. The average colony growing on soybean media was 1.4 cfu/g while the average colony growing on standard media was 1.6 cfu/g. The difference in the number of colonies was due to the composition of the commercial mannitol egg yolk polymyxin standard media which contained peptone,

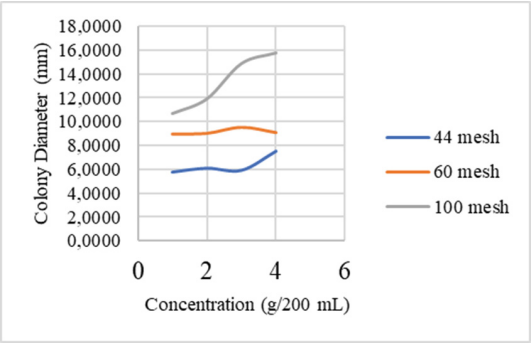

Fig. 4. Effect of Concentration and Size of Soybean Powder on Colony Diameter

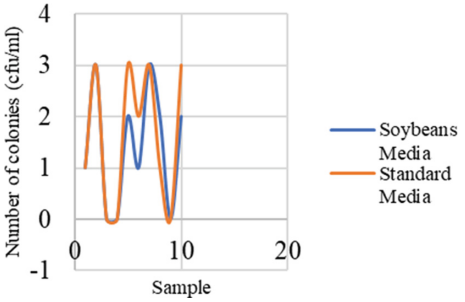

Fig. 5. Comparison of the number of colonies *of Bacillus cereus* on soybean media and standard media

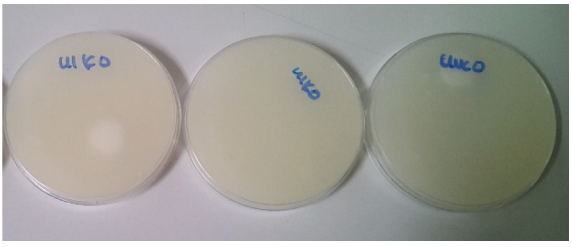

Fig. 6. Bacterial Colonies of *Bacillus cereus* on Soybeans Media

and animal meat extract, which were more easily digested by bacteria than vegetable protein from soybean powder (Fig. 5).

The morphology of bacterial colonies on soybean mannitol egg yolk polymyxin media can be seen in Fig. 6 in round oval shape, opaque white in color and slightly smaller in diameter compared to standard mannitol egg yolk polymyxin media. The average diameter of the bacterial colonies of *Bacillus cereus* on soybean media was 10.36 mm, while on standard MYP media it was 10.46 mm.

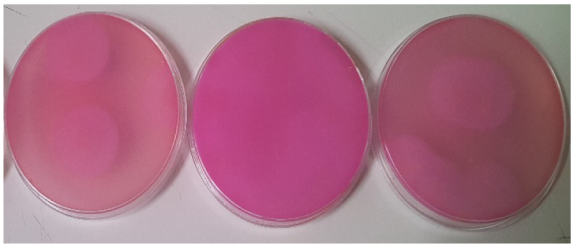

Fig. 7. Bacterial Colonies of *Bacillus cereus* on Standard Media

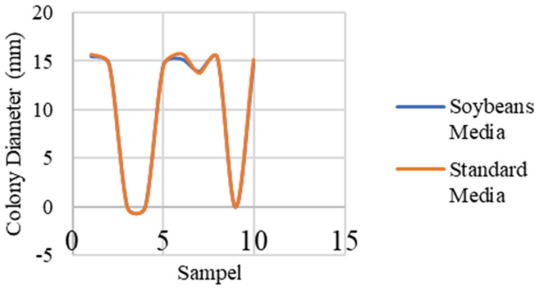

Fig. 8. Comparison of *Bacillus cereus* colony diameter on soybean media and standard media

Colony morphology on standard mannitol egg yolk polymyxin media in Fig. 7 is round and pink in color. This morphological difference was influenced by the adequacy of the media nutrition, the solubility factor of soybean powder, the media dye, and the presence or absence of mannitol fermentation by bacteria. In some samples there was no growth of *Bacillus cereus* bacterial colonies because the creamer samples did not contain *Bacillus cereus* bacterial contamination.

Figure 8 shows that the diameter of the colonies growing on soybean media could match or be equivalent to standard mannitol egg yolk polymyxin media. This also shows that soybean media has almost the same ability or equivalent to standard mannitol egg yolk polymyxin media in growing bacterial colonies of *Bacillus cereus.*

4 Conclusion

Based on the results of the study, it can be seen that the protein content of soybeans is 35.95% so that it can be used as an alternative medium for the growth of *Bacillus cereus* bacteria. Soybeans are a source of high protein for the growth of *Bacillus cereus* bacteria which can replace the presence of meat extract and peptone in standard media. Optimal growth of bacterial colonies of *Bacillus cereus* occurred at a concentration of soy powder 4 g/200 ml and soybean powder size of 100 mesh which was 8.67 CFU/ml. While the optimal bacterial colony size of *Bacillus cereus* occurred at a concentration of 4 g/200 ml soybean powder and a 100 mesh soybean powder size which was 15.74 mm. The comparison test between mannitol egg yolk polymyxin media made from soybeans

and standard mannitol egg yolk polymyxin media made from meat extract showed similar results, namely both media were able to grow *Bacillus cereus* bacterial colonies properly in terms of number, size, and colony morphology. The average colony growing on soybean media was 1.4 CFU/ml while the average colony growing on standard media was 1.6 CFU/ml.

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