

Comparison of Escherichia Coli Bacteria Growth on Soybean Alternative Modification Media

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Abstract: *Escherichia coli* bacterial infections often cause urinary tract infections. Diagnosis of *Escherichia coli* through examination of microscopic, culture, and serological specimens. Examination of culture of *Escherichia coli* requires EMB media containing protein. Soybeans contain 30 grams of protein per 100 grams, thus potentially replacing protein in EMB media. This study aims to determine the growth of *Escherichia coli* in alternative soybean modification media and manufacturer EMB. This study was an experimental laboratory conducted at the Bacteriology Laboratory. Media Alternative modification of soybeans uses a variation of 0%, 25%, 50%, and 100% as a substitute for protein, then each concentration is added lactose 10 grams, dipotassium hydrogen phosphate 2 grams, eosin 2 grams and 15 grams so as compactor, Methylene Blue 0.06 gram as an indicator of fermentation, then planted *Escherichia coli* in each alternative media of soybean modification and manufacturer's EMB as gold standard 370C 24 hours Analysis of the data was described statistically. The results showed that the alternative media modified soybeans in order to replace 100% EMB (Eosine Methylene Blue) media with an average number of *Escherichia coli* colonies grown in this medium as many as 261.5 colonies, almost the same as the average colony on EMB media as a Gold standard of 358 colonies.

Keywords: Soybeans, *Escherichia coli*, Eosine Methylene Blue Activity

1. Introduction

Escherichia coli bacterial infections are often found in people in tropical countries including Indonesia because of the hot and humid climate. *Escherichia coli* is part of the normal flora that adapts well to life in humans, especially in the gastrointestinal tract, turns into a pathogen in the event of changes or decreased endurance of the sufferer. These bacteria become pathogens when they exceed the threshold number. One of the clinical manifestations of *Escherichia coli* infection is an infection of the urinary system. These bacteria can also cause acute and chronic diarrheal diseases. *Escherichia coli* causes disease by attaching to the intestinal mucosa and producing enterotoxins and cytotoxins. The result is mucosal damage, the release of large amounts of mucus which causes diarrhea.

The diagnosis of *Escherichia coli* in the laboratory can be done through examination of microscopic, culture, and serological specimens. Culture is carried out by planting *Escherichia coli* cultures on suitable growth media. Growth media function to isolate, grow, multiply and count the number of microorganisms. Growth media can grow microorganisms if they contain enough nutrients, temperature, appropriate pH, must be sterile, and do not contain inhibitors. Bacteria need nutrients such as water, energy sources, carbon, minerals, growth factors and nitrogen to support their growth [1]. One medium that is suitable and supports the growth of *Escherichia coli* is EMB or Eosine methylene blue. EMB contains peptone, lactose Dipotassium hydrogen phosphate, Eosin, Methylene blue and Agar. Eosin will inhibit the growth of Gram positive bacteria, Peptone is a hydrolysis product of animal or vegetable proteins such as muscle, liver, blood, milk, casein, lactalbumin, gelatin and soybeans. The composition depends on the original material and how to get it. Pepton as a protein source for microorganisms that will be bred.

Methylen Blue as an indicator of lactose & sucrose fermentation. Lactose, allows differentiated gram-negative bacteria based on their ability to ferment lactose.

EMB media ready to use which is sold by factories is relatively expensive, the media can also experience damage if the storage is not considered properly, for the reasons above and the abundance of existing natural resources encourages researchers to find alternative media for bacterial growth from easily available materials and the price is more economical than the factory EMB media. Nutrients contained in the media can be replaced with other ingredients with the same function.

Soy beans (*Glycine max*) or soybeans contain carbohydrates of 30.0 grams per 100 grams, have protein content of 30.2 grams per 100 grams, have 15.0 grams of fat per 100 grams, have phosphorus content of 506 mg per 100 grams, and has a vitamin A content of 41.2 IU per 100 grams. so it is necessary to conduct soybean research in lieu of pepton in EMB medium to *Escherichia coli* growth.

2. Research Methods

Researched growth media is a mixture of materials that function to isolate, grow, multiply and count the number of microorganisms. Microorganisms can grow well if the media contains enough nutrients, the appropriate temperature and pH, must be sterile and not contain inhibiting substances. The necessary nutrients consist of water, energy sources, carbon sources, mineral sources, growth factors and nitrogen. Water is an important component in the medium. Water functions as a solvent and transporter in metabolism. The energy source of microorganisms comes from organic and non-organic materials, especially sunlight. Carbon sources for microorganisms can take the form of organic or inorganic compounds. Mushrooms do not have chlorophyll so they are heterotrophic. Heterotrophic organisms depend on organic carbon sources. Organic compounds include

carbohydrates, fats, proteins and amino acids. Minerals function as cell constituents and regulate osmosis pressure and acidity. The growth factor is an organic compound that is needed for growth as a constituent of cell ingredients. Growth factors are classified as amino acids, as constituents of proteins, as constituents of nucleic acids and vitamins as an active part of enzymes. Vitamin B12 works to help growth. Nitrogen sources can use nitrogen in the form of amino acids and proteins. One of the agar media that is suitable and that supports bacterial growth is EMB. EMB ready-to-use media sold by factories is relatively expensive. Therefore an alternative media is needed to grow mushrooms from materials that are easily obtained and the price is more economical than EMB media. One of the natural ingredients that contains nutrients needed by bacteria for its growth is soybean. Soybean contains protein, carbohydrates, fats, vitamins, calcium, phosphorus, iron, manganese, copper, sodium. Nutrient content contained in red beans is a nutrient content needed by bacteria for growth so that Soybean with the addition of lactose and so that it is expected to grow *Escherichia coli* fungi.

Escherichia coli can grow at an optimum temperature of 37°C, with intervals for growth is between 10°C-40°C with a maximum pH value of 8.5. *Escherichia coli* is a bacterium that is relatively sensitive to heat so that it will die or be inactive at pasteurization temperatures or during food cooking. These bacteria have three antigens including O antigen (somatic), H antigen (flagella), and K antigen (capsule). *Escherichia coli* has biochemical properties, where these germs are able to distribute glucose, lactose, sucrose, mannitol, and maltose by forming acids and gases so that the MacConkey media and the formed eosine methylene blue colony media are pink to dark red with specific metal flashes, and featuring a smooth surface. In the indol and methyl red test the bacteria showed positive results while in the Voges-Proskauer test showed negative results. This bacterium does not hydrolyze urea and does not form H₂S. On the eosin methylene blue media, *Escherichia coli* bacteria showed a distinctive morphological growth in which the growing colonies had rainbow colors that glistened like metal or metallic sheen.

3. Result and Discussion

Laboratory tests are important in making the right casual diagnosis so that we can provide the right medicine. In daily practice, complete laboratory tests are only done if the diarrhea does not heal in 5-7 days.

Laboratory tests that need to be done include examination of stools, namely microscopic and macroscopic, culture of germs, tests of resistance to various antibiotics, pH and sugar levels, if lactose intolerance is suspected. Blood tests are complete blood, electrolyte testing, pH and alkaline reserves (if by giving RL i.v. acidosis is still present), urea levels (to determine the presence of kidney physiology)[2]. Duodenal intubation examination is on chronic diarrhea to look for the germs that cause.

Soybean Research Results Data. as an alternative media modification substitute for EMB (Eosine Methylene blue) Against *Escherichia coli* Growth.

Analytic Test

Significant value of the results of the count of 0,000 with alpha (0.05), means that there is an effect of soy concentration on alternative media modification of Soybean Agar on the number of *E. coli* colonies, or the higher the Soybean concentration. dissolved the higher the number of *E. coli* colonies. The results of *Escherichia coli* growth data on EMB media and alternative media for soybean modification in order to meet the requirements to carry out the One Way Anova test that is normally distributed data and homogeneous data variants which means there is a difference in the growth of *Escherichia coli* in the EMB media with alternative media modified Soybean Agar.

The results of macroscopic examination of the shape and color of *Escherichia coli* colonies on EMB media and alternative media as well as microscopic observations of pure culture of *Escherichia coli*, showed positive results of *Escherichia coli* correctly. Macroscopic examination showed a green metallic colony which was very typical after incubation at 37°C for 24 hours, while for microscopic examination of Gram showed a short red fat stem.

Eosine Methylene Blue can be used to isolate *Escherichia coli* bacteria. The results showed that *Escherichia coli* grew on EMB media which was shown by the average yield of colonies growing as many as 358 colonies, because in the EMB media it contained enough nutrients for *Escherichia coli* growth with a composition consisting of peptone, lactose, Eosine, Methylene Blue and so that. Eosin Methylene Blue media supports the growth of *Escherichia coli* because Eosine can inhibit the growth of gram-positive bacteria which also require a neutral environment with a pH of 7.0, and the optimum temperature for growth between 25-30 ° C Pepton as a protein source for microorganisms to be cultured. On the media Eosin Methylene Blue: as an indicator of lactose fermentation. Lactose, allows differentiated gram-negative bacteria based on their ability to ferment lactose

Escherichia coli growth in alternative media modified Soybean Agar

Alternative media modification of Soybean Agar. an EMB modification medium consisting of protein derived from soybeans, lactose, hydrogen phosphate dipotassium, eosin, Methylene Blue as an indicator of lactose fermentation and agar as a compactor. For growth, microorganisms need nutrients which include carbon, nitrogen, non-metallic elements such as sulfur and phosphorus, metal elements such as Ca, Zn, Na, K, Cu, Mn, Mg, and Fe, vitamins, water, and energy). Soybeans also contain several nutrients, including every 100 grams containing 30.0 grams of carbohydrates, 30.2 grams of protein per 100 grams, 15.0 grams of fat 506 mg of phosphorus, vitamin A 41.2 IU per 100 grams.

Carbohydrates as the main source of Carbon, Hydrogen, and Oxygen, while protein is the main source of Nitrogen [3]. Alternative media for modifying soybeans In order to use EMB (Eosine Methylene Blue) media to grow *Escherichia coli*, it can be used. Based on research that has been done, with several variations of Soybean concentration. which is made into agar by adding Bacteriological so that as a

compactor the different growth results of the Escherichia coli colony are obtained. The results of the number of Escherichia coli colonies growing on soybean modification alternative media can be seen in table 4.1. The table illustrates that the results of the number of Escherichia coli colonies growing on alternative media modified Soybean Agar with a concentration of 0% does not indicate the growth of Escherichia coli colonies. This is because the media contains only lactose, dipotassium hydrogen phosphate, eosin, and so as compactors, Methylene Blue as an indicator of fermentation so that it does not meet the nutritional needs of Escherichia coli to do its growth. In an alternative media modification of Soybean Agar with a concentration of 25% there is a growth of Escherichia coli colonies with an average number of colonies as much as 82.5 (83)[4]. This is because the alternative media modification for Soybean Agar contains as much as 25% soybeans, so that the bacteria are able to grow with the source of nutrients contained in the media. In alternative media modification of Soybean Agar with a concentration of 50% there is growth of Escherichia coli with an average number of colonies of 161.8 (162). This is caused by as much as 50%, so the number of Escherichia coli colonies that grows more when compared to alternative media modification of Soybean Agar at 25% soybean concentration because bacteria are better able to do their growth with the nutritional sources contained in the media. In alternative media modification of Soybean Agar with 100% soybean concentration there is growth of Escherichia coli with an average number of colonies of 261.5. This amount is greater when compared to alternative media modification of Soybean Agar at a concentration of 50%, even nearly equaling the results of Escherichia coli growth on EMB (Eosine Methylene Blue) media as the Gold Standard. This is due to the alternative media modification of Soybean Agar that contains 30 grams of protein, so that bacteria have very adequate nutrients to carry out its growth. With the results showing the growth of Escherichia coli, it can be said that alternative modification of soybean agar contains nutrients needed by germs for its growth, but only on alternative media modified soybeans with a concentration of 100% that can be used as an alternative growth medium of Escherichia coli because the number of Escherichia coli colonies growing can approach the number of Escherichia coli colonies growing on EMB (Eosine Methylene Blue) media as the Gold Standard

4. Conclusion

The alternative media modified soybeans in order to replace 100% EMB (Eosine Methylene Blue) media with an average number of Escherichia coli colonies grown in this medium as many as 261.5 colonies, almost the same as the average colony on EMB media as a Gold standard of 358 colonies

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