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Identification of Escherichia Coli Bacteria in Tofu Soaking Water Sold at Bersehati Market Manado City North Sulawesi Province

Tania Jennet Salaila[1], Ferny Margo Tumbel[2], Helen J. Lawalata[3], Meity N. Tanorl[4], Verawati I. Y. Roring[5]

[1,2,3] Department of Biology, FMIPAK, Universitas Negeri Manado, Indonesia

e-mail: tjennetsalaila@gmail.com

Abstrak

Produk tahu sudah lama menjadi makanan pokok masyarakat Indonesia, terlebih khusus di Kota Manado. Selain harganya murah produk tahu juga sangat banyak dijumpai di tempat-tempat perbelanjaan tradisional maupun swayalan. Tahu memiliki banyak manfaat sebagai sumber protein pengganti dari ikan dan daging. Pada waktu pembuatan tahu biasa kita rendam dengan air terlebih dahulu, karena tahu banyak mengandung protein air rendamannya harus higienis, karena jika tidak higienis akan berpotensi sebagai sarang bakteri pembawa penyakit. Penelitian ini mengenai identifikasi bakteri E.coli pada air rendaman tahu yang diperoleh dari Pasar Bersehati Kota Manado. Air rendaman tahu sudah banyak diteliti, namun belum banyak pengujiannya menggunakan Uji Sitrat, Uji SIM, Uji MR-VP. Penelitian ini bertujuan untuk mengidentifikasi bakteri E.coli di rendaman air tahu. Rancangan penelitian yang digunakan adalah rancangan penelitian deskriptif, mikroskopik, pewarnaan gram, uji biokimia. Hasil penelitian menunjukkan bahwa air yang diambil dari 5 sampel menunjukkan adanya 5 isolat bakteri patogen. Berdasarkan media tumbuh bakteri hanya 3 isolat yang teridentifikasi bakteri E.coli melalui isolasi, pengenceran, pewarnaan gram, dan uji biokimia. Sedangkan 2 sampel tidak teridentifikasi bakteri E.coli. 5 sampel yang telah diteliti ternyata hanya sampel 1,2, dan 4 yang teridentifikasi bakteri E.coli sedangkan sampel 3 dan 5 tidak teridentifikasi bakteri E.coli.

Kata Kunci: *Bakteri E.coli, Air Rendaman Tahu, Pasar Bersehati, Biologi*

Abstract

Tofu products have long been a staple food for Indonesian people, especially in the city of Manado. Apart from being cheap, tofu products are also often found in traditional and traditional shopping places. Tofu has many benefits as a source of protein to replace fish and meat. When making tofu, we usually soak it in water first because tofu contains much protein. The soaking water must be hygienic because if it is not hygienic, it has the potential to become a nest for disease-carrying bacteria. This research concerns the identification of E.coli bacteria in tofu-soaking water obtained from Bersehati Market, Manado City. Tofu soaking water has been researched a lot, but only a little has been tested using the Citrate

Test, SIM Test, and MR-VP Test. This research aims to identify E.coli bacteria soaked in tofu water. The research design used was descriptive, microscopic, gram staining, and biochemical tests. The results showed that the water taken from 5 samples showed the presence of 5 isolates of pathogenic bacteria. Based on the bacterial growth media, only 3 isolates were identified as E. coli bacteria through isolation, dilution, gram staining, and biochemical tests. Meanwhile, 2 samples did not identify E.coli bacteria. Of the 5 samples that were examined, it turned out that only samples 1, 2, and 4 were identified as E. coli bacteria, while samples 3 and 5 were not identified as E. coli bacteria.

Keywords: *E.coli bacteria, Tofu soaking water, Bersehati Market, Biology*

INTRODUCTION

Water is the largest chemical element in food and is an essential part of the life of living things. Water can affect the texture and taste of food products. Water content has a significant influence on microbiological activity, which can damage a food product during storage and transportation. The water consumed by the public must meet health requirements because water is an excellent medium for the development of microbes. Water management is used to obtain water that meets the requirements, namely the stages during storage, the filtering process, and chlorination. Water can be used for the process of managing food products, but the water used is clean water that is not contaminated by microbes. One of the many food products that require water in the management process is tofu (Chandra, 2007).

One of the many food product communities that produce vegetable protein is known to local people as soybeans. In the food plant community, soybeans are the third essential group after rice and corn (Tangendjaja & Ilham, 2003). Soybeans are a group of secondary crops that contain a lot of protein. Soybeans have a role as a source of vegetable protein, which is very essential in order to improve population nutrition because apart from being safe for health, soybeans are also relatively cheap compared to sources of animal protein (Ditjentan, 2004).

Tofu is one of the many food products whose source is protein, ingredients based on soybeans, which are very popular with the Indonesian people. Generally, tofu products in Indonesia are produced by small-scale entrepreneurs who are generally found on the island of Java, especially in Semarang. The tofu industry is experiencing very rapid development, which is in line with the increase in the number of people in Indonesia.

Tofu is easily damaged and destroyed. At room temperature, it can last for 24 hours to 48 hours. After this time limit, the taste of the tofu becomes sour, and the color, aroma, and texture of the tofu will change so that it can no longer be consumed. This is due to the relatively high water and protein content of tofu, 86% in each tofu. Tofu has a fat content of 4.7% and carbohydrates of 1.7%. Due to its composition, tofu is a food product that is suitable as a medium for the growth of spoilage microbes, especially pathogenic microbes (Koswara, 2011).

In the city of Manado, North Sulawesi province, you often find sellers selling tofu. The tofu they sell is soaked in water to soak the tofu and maintain its texture. However, traders

often don't care about the quality of the water used to soak the tofu. When managing tofu or during the process of storing tofu, it is necessary to use clean water because if the water used does not meet the specified requirements, it can cause the growth of pathogenic microbes. According to the Republic of Indonesia Minister of Health Regulation No. 17/MENKES/PER/IX/2009 regarding the requirements and quality of clean water which determines that Escherichia coli in clean water is 0/100 mL of the sample of bacteria. Escherichia coli is a microorganism that generally lives normally in the intestines of living creatures. If Escherichia coli is found at 0/100 mL in the sample, then there is a big chance that Escherichia coli will be present, and its use for drinking water should be reconsidered because the water or food has a high chance of being contaminated by dirty materials.

Providing food products that are hygienic and in accordance with established health standards is the initial principle of providing institutional food products. Food products that are not managed properly by buyers or sellers of these food products can result in negative impacts, for example, infections and poisoning of human internal organs (Fatmawati, 2014). The cause of contaminants in food products is generally pathogenic bacteria, one of the many pathogenic bacteria, namely the microbe Escherichia coli.

These microbes are originally found in the feces of living creatures and are transmitted to food products due to someone who has handled food unhygienically, cooking utensils or food utensils that have not been cleaned sterilely, the health of the seller or buyer of food products and the use of washing water that has been contaminated. Contaminated with Escherichia coli (Susana, 2003). Escherichia coli is a microbe that contains bacteria and negative salts that live in the digestive tract, such as the intestines, both in living creatures. Escherichia coli can contaminate food products, so the presence of pathogenic microbes in food ingredients or raw food indicates the emergence of a health threat to consumers (living creatures) because it can be said that the food product has been contaminated by pathogenic microbes. Escherichia coli microbes are microorganisms that generally live in the organs of living things and are known to help vitamin K, which is essential for blood clotting (Entjang, 2000)

Management of food products must be monitored to guarantee food products and also to prevent the spread of disease from food products, including chronic diarrhea resulting in bleeding, vomiting, and meningitis (Purnawijayawanti, 2001). Diarrhea is a condition characterized by a soft or liquid consistency, so it can be like water in more frequent amounts than usual (three times more) a day (Ministry of Health of the Republic of Indonesia, 2011). Diarrhea can be caused by abnormal transportation of water and electrolytes in the intestines. Throughout the world, as many as 600 million children experience diarrhea every year, and 21% of all deaths in children living in developing countries are due to diarrhea and dehydration. Diarrhea involves the stomach, colitis, and the large and small intestines (Wong, 2008).

Previous research on pathogenic microbes that contaminate food products, including research (Malsin, 2016) regarding the identification of E.coli microbes in tofu sold at the Panjang Bpngoeya market, Wua-Wua District, showed that there were six tofu sellers, or 31%, who tested positive for selling tofu that had been contaminated. E. coli microbe. According to scientific research (Yanti Rosila, 2006), 5 out of 12 samples of tofu marinade

sold at the Bagan Batu market are in accordance with established health standards (0 in 100 ml of sample). And seven other samples did not comply with established health standards (more than 0 in 100 ml of sample).

Based on the many possibilities for Escherichia coli microbes to live and grow in food, this research was carried out with the title "Identification of Escherichia coli bacteria in tofu soaking water sold at Bersehati Market, Manado City, North Sulawesi Province."

METHOD

This type of research is carried out using a descriptive method and takes into account the number of microbial colonies using the TPC (Total Plate Count) technique. Research samples will be taken at the Bersehati Market, Manado City, and this research will be carried out on 17 March 2022 - 27 August 2022 in the Microbiology laboratory of the Department of Biology Faculty of Mathematics Sciences Nature and Geosciences State University Manado in Tondano, North Sulawesi. The research population includes tofu sellers who sell at Bersehati Market, Manado City. Meanwhile, the samples taken were soaked in tofu water from five (5) different tofu sellers, then the samples were diluted into sterile distilled water with dilutions of 10^{-1}, 10^{-2}, 10^{-3},10^{-4},10^{-5},10^{-6}, and 10^{-7}.

The tools used in this research were sterile tissue, petri dishes (Pyrex), L rods (drygalsky), test tubes (Pyrex), measuring cups (Pyrex), beakers, aluminum foil, vernier calipers (tricle), dropper pipettes (Brand), tube needle (Mico), lab spatula (sellaco), cover glass (OneLab), plastic wrap, micropipette (Eppendorf) and tip (Sakamed), analytical balance (Fujitsu), shaker (Oregon), knife, oven (Memmert), incubator (Memmert), autoclave (Gea), hotplate (Velp), laminar airflow cabinet (B-One), magnetic stirrer (Basco), Bunsen, microscope and Erlenmayer flask (Pyrex). The materials used in this research include Eosin Methylene Blue (EMB) media, 70% alcohol, 96% alcohol, distilled water, and tofu soaking water from Bersehati Market, Manado City, North Sulawesi Province.

This research consists of the following stages: preparation stage (sterilization of tools and materials and preparation of medium), dilution of tofu soaking samples, isolation of bacteria from dilutions 10^{-1}, up to 10^{-7}, microbiological tests of morphology and physiology of bacteria and biochemical tests of bacteria from tofu soaking water from Pasar Bersehati Kota. Manado, North Sulawesi Province.

Tools and materials used in research will undergo sterilization to prevent contamination during testing. Initially, the equipment to be used is sterilized, followed by a drying process. Next, the sterilized instruments and their media were sterilized using an autoclave at a temperature of 121˚C for a duration of 15 minutes. Usually, the instruments that undergo sterilization by autoclaving are glassware, including test tubes, Erlenmeyer flasks, and Petri dishes. Alternatively, additional tools can be sterilized by lighting a Bunsen lamp or soaking them in alcohol and exposing them to the heat of a Bunsen flame.

Making EMB media is used to isolate and purify E.coli bacteria, screen for antibacterial E.coli bacteria, and handle bacterial tests. The stages of the procedure are as follows: weigh 23.5 grams of the instant medium, suspend it in distilled water, make the final volume 1000 mL, and then heat this suspension until the agar is cooked. Then, put it in a test tube (± 5 mL for inclined media and ± 10 mL for upright media) and sterilize it using an

autoclave at a temperature of 121ºC and a pressure of 2 Atm. For 15 minutes, store it in a cool and dry place in the refrigerator (Rossita, 2015).

Isolate was obtained from tofu soaking water at Bersehati Market, Manado City, North Sulawesi Province; 10 mL of tofu soaking water was taken and then added to 9 mL of distilled water solution. Isolates were incubated for 24 hours at 37 ˚C. Next, a dilution series of 10-1 to 10-7 was made, then inoculated on Eosin Methylene Blue (EMB) media and incubated at 37 ˚C for 24 hours.

Purification of bacterial isolates is carried out by transferring the bacteria using the line method, which is then grown. Purification aims to obtain the desired pure culture without any other microbes. The selection of purified microbial colonies is based on differences in the morphological appearance of the colonies, both in terms of color, elevation, surface texture, radial lines, concentric circles, and exudate drops so that pure isolates are obtained on EMB media.

In this research, data analysis was carried out to determine the identification of E.coli bacteria, then it was described descriptively in the form of a table so that it could describe how many mycorb colonies were obtained from the tofu soaking water, and it was described descriptively for the results of the development of bacterial colonies in specific media and biochemical tests.

RESULT AND DISCUSSION

Result

1. Microbial Isolation Results using the TPC (Total Plate Count) Method

Samples taken at Bersehati Market, Manado City, were five samples of tofu soaking water. Sample 1 was taken from a seller at the north front of the market. Sample 2 was taken from a seller at the north center of the market. Sample 3 was taken from a seller at the west front. , Sample 4 was taken from a seller precisely in the west-central part, and Sample 5 was taken from a seller precisely in the back east of the market. Tofu-soaking water samples were isolated based on the dilution method using a sterile distilled water solution (Figure 1). Next, a dilution series of 10-1 to 10-7 was made, inoculated on Eosin Methylene Blue (EMB) media, then incubated in an incubator at 32℃ and incubated for 24 hours. EMB media is used to isolate and differentiate enteric microbes or coliforms. Isolation results of tofu-soaking water samples obtained five bacterial colonies. The growing bacterial colonies are then purified to obtain pure isolates according to the colony morphology.

Figure 1. Sample of tofu soaking water taken from Bersehati market, Manado City, North Sulawesi

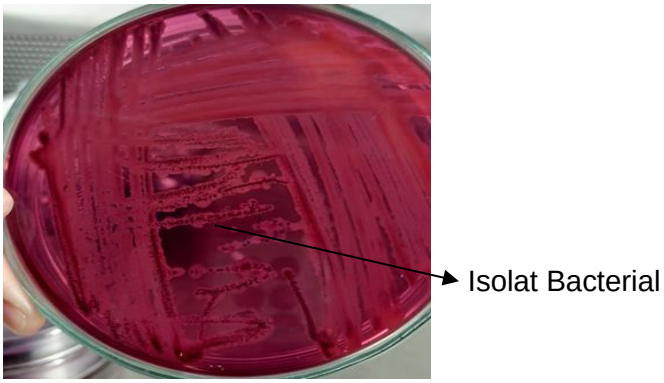


Figure 2. E.coli bacteria on EMB media

Bacterial colonies that grow on EMB media are irregularly round and purplish pink in color. The bacteria inoculated on EMB media showing purplish pink colored colonies are Escherichia coli bacteria, and the pink colored colonies are thought to be Klebsiella sp and Enterobacter aerogenes bacteria (Brooks, 2012).

2. Identification of Bacterial Colony Morphology

In the results of bacterial isolation, there is a variety of bacterial colony morphologies including colony shape and colony color. The following results can be presented in the form of Table 1.

Table 1. Bacterial Colony Isolation Results from tofu soaking water samples

Sample	Form colonies	Bacterial Colony
S 1	Round	Pink Purple-purple
S 2	Round	Pink Purple-purple
S 3	Round	Pink
S 4	Round	Pink Purple-purple
S 5	Round	Pink

Colony color after incubation for 1 day in a petri dish. The results of the morphology of indigenous bacterial isolates can be seen in table 1.

3. Biochemical Characterization of Bacterial Cells

Bacteria characterized morphologically (gram staining) are then characterized biochemically. Biochemical tests carried out were citrate fermentation, indole sulfate, motility, and Methyl Red-Voges Proskauer. Gram-positive bacteria can be distinguished by their ability to retain the crystal violet stain even after exposure to safranin. However, gram-negative bacteria can be identified by their inability to retain the primary stain when rinsed with alcohol, resulting in a reddish appearance after safranin staining. The results of Gram bacterial staining obtained from water samples used to soak tofu can be seen in Table 2.

Table 2. Bacterial Gram Staining Results from tofu soaking water

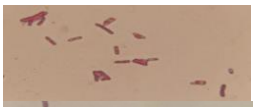
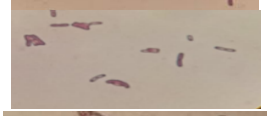
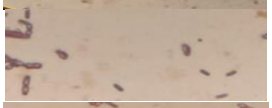
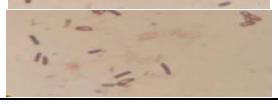
Isolate Code	Isolate Image	Gram Staining Results
Sample 1		Negative
Sample 2		Negative
Sample 3		Negative
Sample 4		Negative
Sample 5		Negative

Table 3. Results of Biochemical Characterization and Morphology of Bacterial Cells from Tofu Water Soaking

Isolate Code	Colony Morphology: Cell Shape	Colony Morphology: Cell Arrangement	Gram	Sitrat	SIM	MR-VP	Bacteria
Sample 1	Basil	Pair	-	+	+	+	*Escherichia coli*
Sample 2	Basil	Single	-	+	+	+	*Escherichia coli*
Sample 3	Basil	Pair	-	-	-	-	*Klebsiella*
Sample 4	Basil	Single	-	+	+	+	*Escherichia coli*
Sample 5	Basil	Pair	-	-	-	-	*Enterobacter*

4. Calculation of the Number of Bacterial Colonies

At this stage, each sample was taken twice and each concentration was planted twice in EMB media. The colonies that grew in each collection and dilution were calculated on average for each sample until the results of microbial growth were obtained in Table 4.

Table 4 Colony Counting Results in Each Sample

Sample

Concentration	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
10^{-1}	106	99	102	118	125
10^{-2}	83	78	87	93	95
10^{-3}	65	43	35	67	41
10^{-4}	3	1	0	11	5
10^{-5}	0	0	0	0	0

Based on Table 4, the results of colony calculations in each sample of tofu soaking water were obtained and in order to know the number of microbes in each sample, calculations were carried out using the colony calculation formula to obtain the results of microbial calculations in each sample of tofu soaking water which can be seen in Table 4.

Table 4. Calculation of the Number of Colonies in Each Sample Using the TPC Method

Sample	Average number of colonies (CFU/mL)	Information
S1	$2{,}61 \times 10^5$	Exceeds threshold
S2	$1{,}37 \times 10^4$	Do not exceed the threshold
S3	$1{,}12 \times 10^4$	Do not exceed the threshold
S4	$2{,}30 \times 10^5$	Exceeds threshold
S5	$2{,}54 \times 10^5$	Exceeds threshold

Discussion

Isolation of bacteria from tofu soaking water was carried out using the 10^{-1} to 10^{-7} dilution method. Next, pour 1 mL of the bacterial dilution using the pour plate method into Eosin Methylene Blue Agar (EMBA) media. EMB media is a culture medium used to differentiate fecal and non-fecal coliform bacteria based on their ability to ferment lactose. Coliform bacteria are microorganisms. It is a collection of rod-shaped Gram-negative bacteria that do not produce spores. They have the ability to survive with or without oxygen, do not produce oxidase enzymes, and are able to break down lactose into acid and gas within a period of 24 to 48 hours at a temperature of 35 degrees Celsius. Group 4 coliform bacteria are classified as opportunistic pathogens (Carroll, Brutel, & Morse, 2015; Halkman & Halkman, 2014; Murray, Rosenthal, & Pfaller, 2020). The coliform bacteria group consists of the genera Citrobacter, Enterobacter, Escherichia, and Klebsiella (Halkman & Halkman, 2014; Tominaga & Ishii, 2020).

EMB media is a nutrient-rich medium designed to support the growth of lactose fermenting bacteria. The ingredients consist of: (a). Peptone contains 10 grams of nitrogen, minerals, amino acids and vitamin B. Lactose contains 5 grams of carbohydrates which can be fermented by microorganisms. Eosin, with a concentration of 0.4 grams, is used as a pH indicator and inhibits the growth of gram-positive bacteria. Methylene blue with a concentration of 0.065 grams functions as a pH indicator, the same as eosin (E). 5 grams of

sucrose is used to supply carbohydrates and differentiate colonies of Coliform Bacteria and non-Coliform Bacteria. Woman. 2 grams of K2HPO4 (dipotassium phosphate) provides electrolytes and helps maintain osmotic balance. The user text is "(G)". Agar-agar which is usually used in solid media acts as a consolidator (Akhwan, 2017). The single isolate that had grown on EMB media was taken and the results of this stage obtained 5 isolates, namely 2 isolates from the 10^{-4} dilution and four isolates from the 10^{-5} dilution. The five isolates were then purified twice, the purified isolates were then made into stock for the next research stage. After the isolation stage, the next stage is the stage of identifying bacterial isolates which is done by observing the morphology of the bacteria which appears in the form of color, shape, edges and surface. So the result of this stage is to characterize the morphology of the five (5) bacterial colonies. The results of colony characterization were based on colony color, colony edges, and colony elevation in the 5 colonies (Table 1).

The results of the isolation and identification of E. coli bacteria that grow on EMB media show that sample 1, sample 2 and sample 4 of tofu soaking water contain E. coli bacteria with colonies that have a purplish pink color. This is because these microbes can break down lactose quickly. Gram-negative bacteria that can ferment lactose (usually microbes in the intestine) can produce acid, in acidic conditions they can produce a complex color, purplish pink or metallic green. Based on Risna Putri's research, colonies that are still purplish have smooth and even purple edges, and a pink center with a hint of green. Khakin and Rini (2018) stated that this special characteristic is still associated with E.coli bacteria.

Sample 3 and Sample 5 are suspected to contain Enterobacter and Klebsiella bacteria. Enterobacter bacteria show a purplish pink color, with some strains showing a strong purple color. Purplish pink colonies show similar characteristics to dark purple colonies, in particular having irregular but distinct edges, a dark and smooth center, and a flat feel. Zahrotu (2016) stated that this colony characteristic is usually observed in Enterobacter sp. The Klebsiella bacteria in the study are believed to have a pink or purplish pink color, which is similar in appearance but much darker. Pink bacterial colonies have a distinct flatness along the edges and a somewhat higher elevation structure. Sari et al (2019) classify it as a member of the Coliform Klebsiella bacteria group.

Based on the results of research conducted by Malsin (2016) regarding the identification of E.coli bacteria in tofu sold at the Panjang Bonggoeya Market, Wua-wua District, Kendari City, it shows that 31% of 64 tofu traders tested positive for selling tofu contaminated with E.coli bacteria. Research conducted by Jayatno (2016). Yanti Rosila's investigation in 2006 found that only 5 of 12 samples of tofu water supplied by street vendors at Bagan Batu market met health requirements, and there were no traces of contaminants in a 100 ml sample. Apart from that, 7 other tofu water samples did not meet health standards, namely more than 0 in 100 ml of sample. Susanna, et al (2003, p. 22) stated that E.coli bacteria is a contamination that is often detected in food. This bacteria comes from human and animal waste and is transmitted to food due to unhealthy handler practices, inadequate cleaning of equipment, health conditions of food processors and handlers, and the use of water for washing that contains E.coli. Water pollution can cause several diseases such as Diarrhea, Hepatitis A, Lead Poisoning, Cholera, Amoebiasis, Dysentery and Trachoma (Suriawira, 1996). According to data from the World Health Organization (WHO), around 13

million people die every year from diseases caused by water contaminated with E. coli bacteria (Atmaja, 2009). Indonesia has a prevalence of diarrhea caused by water pollution, which affects around 423 out of every 1000 people of all age groups.

Based on the results of observations through a microscope, gram staining results were obtained from E. coli microbes, which have round cells (cocci) and a single arrangement and have gram negative characteristics. Meanwhile, the microbe Klebsiella sp. has a rod cell shape, has a pairwise arrangement, has negative characteristics.

In (Table 3) the biochemical test used is the IMVIC test. The imvic test is an abbreviation for the Indol, Methyl Red, Voges Proskuer, and Citrate test (BPOM RI, 2008). This test was carried out in order to determine the nature and metabolism of microbial cells that grow in EMB and SSA media by observing the potential of bacteria in fermenting carbohydrates, producing indole, producing gas, and producing acids and others. According to Mahon (2015), E. coli bacteria usually produce positive indole results, negative citrate results and positive MR-VP test results. Based on the results of observations, it showed that the three isolates were motile active, indicated by the presence of a red ring around the SIM medium. According to (Cappuccino & Sherman, 2005) if a positive result is indicated, it is indicated by the presence of motile activity from the bacteria which makes the SIM medium appear as a needle puncture mark. Based on the results of observations, it was found that sample 1, sample 2 and sample 4 were weak in utilizing citrate as the sole carbon source because there was no color change in the medium to blue, whereas isolates of Sample 3 and sample 5 were able to utilize citrate as the sole carbon source. because it was found that there was a color change in the medium which started from green to blue. Biochemical tests were carried out on E. coli using the IMVIC test, to show that E. coli microbes and other bacteria have almost the same characteristics, namely Klebsiella and Enterobacter (Fardiaz, 1992).

CONCLUSION

Based on the research results, Escherichia coli bacteria were found in samples 1, 2, and 4. Meanwhile, samples 3 and 5 did not contain Escherichia coli isolates. Based on these findings, it is recommended to conduct an evaluation of vendor hygiene, environmental sanitation, food processing, storage, and food serving practices to identify specific variables responsible for significant bacterial contamination in food. In future research, it is recommended to only use the MPN approach to increase ease of implementation.

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