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IDENTIFICATION OF Escherichia coli AND Salmonella sp. ON SOME PLANTATION PRODUCTS

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Abstract

Identification of Escherichia coli and Salmonella sp. In Several Plantation Products, the aim is to explain the results of the identification test of E. coli and Salmonella sp. on plantation products. The research was carried out with 4 treatments and 2 replications. Each replication consisted of 5 dilutions. The treatments used were cocoa powder, compost, water and lettuce in selective growing media (EMBA and SSA). The parameters observed were to see whether there were E. coli or Salmonella sp. that grew in the four samples, the number of colonies and color changes that occurred as well as gas or bubbles produced in the fermentation test of sugars (glucose, sucrose and lactose). Based on the test results, it was found that E. coli and Salmonella were not present in the cocoa powder samples in EMBA and SSA media. In the compost sample, the bacteria E. coli grew on EMBA media, but Salmonella bacteria did not grow on SSA media. Lettuce samples showed E. coli growing on EMBA media, while on SSA media there was E. coli and Salmonella bacteria. Meanwhile, in water samples, E. coli, Salmonella and other bacteria grew on EMBA media. And on SSA media, Salmonella and Shigella bacteria grow. In the fermentation test of sugars such as glucose, sucrose and lactose, the media changes color to yellow and there are gas/air bubbles. This shows that bacteria can ferment carbohydrates and can produce gas. Salmonella and other bacteria grow on EMBA media. And on SSA media, Salmonella and Shigella bacteria grow. In the fermentation test of sugars such as glucose, sucrose and lactose, the media changes color to yellow and there is gas/air bubbles. This shows that bacteria can ferment carbohydrates and can produce gas. Salmonella and other bacteria grow on EMBA media. And on SSA media, Salmonella and Shigella bacteria grow. In the fermentation test of sugars such as glucose, sucrose and lactose, the media changes color to yellow and there is gas/air bubbles. This shows that bacteria can ferment carbohydrates and can produce gas.

Keywords: **Escherichia coli, Salmonella sp., Selective Media, Sugar Fermentation Test**

1. INTRODUCTION

Most people pay less attention to the hygiene of the food or drink they buy, so that the circulating food and drink can cause food borne disease. Foodborne illnesses caused by bacteria can be in the form of intoxication or infection. Intoxification through food is caused by the presence of bacterial toxins that are formed in food when bacteria multiply. While infection through food is caused by the entry of bacteria into the body through contaminated food and the body reacts to these bacteria. According to Suprapto, (2008) foodborne disease can be caused by various types of microbes, such as coliforms. Microbes including coliforms and the most commonly causing infections in food are E. coli and Salmonella sp.

Escherichia coli is a normal flora of the digestive tract, but can become pathogenic if the number increases or is outside the digestive tract. If E. coli is present in water or food containing water, it is an indication that the water is contaminated with feces. Coliforms are microorganisms that are present in large amounts in the feces of humans, fish, mammals and poultry. These bacteria

are often used as an indicator of fecal contamination and poor sanitation conditions for water, food, milk and dairy products (Masduki, 1996). While Salmonella sp. are pathogenic bacteria in the digestive tract. Often Salmonella sp. contaminates meat, because the water and protein content in meat is a lot to support the growth of bacteria. According to Noah,

Cases of poisoning that occur in humans originating from foods such as meat or other food ingredients can occur as a result of the way they are processed. Processing that has not reached perfection causes microbes to produce toxins that can damage food and people who consume these foods (suffer from digestive disorders). So that the safety of these foodstuffs also needs to be tested for microbial contamination in the laboratory.

Basically, poultry manure also contains several microorganisms including pathogenic bacteria such as E. coli and groups of bacteria found in the digestive tract of animals. Thus, from the biological aspect and the level of environmental hygiene, fertilizers derived from animal waste can cause concern. Testing the quality of organic fertilizers derived from animal manure, whether poultry, goats, cows or bat-type mammals, is very important. Pathogenic microbes tested based on the quality standards of organic fertilizers derived from animal manure on the minimum technical requirements for solid organic fertilizers are microbial contaminants such as E. coli and Salmonella sp.

Based on this background, this test was designed to identify the presence of pathogenic bacteria from the faecal group of E. coli and enteric bacteria such as Salmonella sp. on cocoa powder (packaged), compost, water (fill and well) and fresh vegetables (such as lettuce) using bacterial isolation and identification techniques.

2. IMPLEMENTATION METHOD

This activity was carried out at the Microbiology Laboratory of the Research Center for Biological Resources and Biotechnology, LPPM IPB (Bogor Agricultural University), Bogor on 07–11 November 2016. The materials used in this activity are bacterial growth media such as EMBA, SSA, bacteriogical agar (agar biotech), aquades, NaCl, 0.45 m milipor paper, cocoa powder, compost, refill water, well water, fresh vegetables (lettuce), sugars (glucose, sucrose and lactose) biochemical test materials, cotton.

EMBAs are selective media and differential media. This medium contains Eosin and methylene blue, which inhibit the growth of Gram positive bacteria, so this medium was chosen for Gram negative bacteria. EMBA also contains lactose carbohydrates, in the presence of lactose carbohydrates bacteria can live in it.

The test was carried out on 4 (four) treatments and 2 (two) replications. Each replication in each treatment consisted of 5 (five) dilutions. The treatments used in this test were cocoa powder, compost, water and lettuce on each selective growing medium (EMBA and SSA).

3. RESULTS AND DISCUSSION

3.1 Identification of Bacteria Growing on Selective Media

Identification of E. coli and Salmonella sp. on some samples (cocoa powder, compost, water and lettuce) in selective growing media (EMBA and SSA) can be seen in Tables 2, 3, 4 and 5.

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1. Sample I (Cocoa Powder)

Table 1 Identification of E. coli and Salmonella sp. on sample I (cocoa powder) with multiple dilutions of 1 his

No	Dilution	Test			
		EMBA		SSA	
		1	2	1	2
1	10-1	-	-	-	-
2	10-2	-	-	-	-
3	10-3	-	-	-	-
4	10-4	-	-	-	-
5	10-5	-	-	-	-

Table 1 shows that both E. coli and Salmonella bacteria did not grow on each of the selective media the day after isolation. Even though it is known that bacteria will germinate after 1x24 hours, especially if the temperature for growth supports that is 37°C. This means that the two bacteria were not present in the cocoa powder samples, because the bacteria had died during several sterilization processes until they were packaged. Several stages of cocoa bean processing according tohttp://www.iccri.net/pengolahan-cocoa/namely the fermented cocoa beans are mechanically dried (drying stage) at a temperature of 50-55 °C. The roasting stage is carried out at a temperature of 115-120°C for 20-30 minutes. Koncing process which evaporates the remaining water and compounds that cause flavor defects, using a temperature of 60-70°C for 18-24 hours. Meanwhile, according to Anonimus (2008), Salmonella is sensitive to heat and will be killed by heating evenly (above 70°C).

Proper processing and hygienic handling of food can prevent Salmonella infection. Similarly, what Hawa (2011) said that E. coli was inactivated above a temperature of 40-45°C.

2. Sample II (Compost Fertilizer)

Table 2 Identification of E. coli and Salmonella bacteria in sample II (compost fertilizer) with multiple dilutions of 1 his

No	Dilution	Test			
		EMBA		SSA	
		1	2	1	2
1	10-1	□	-	E. coli	-
2	10-2	□	□	-	E. coli
3	10-3	□	□	-	-
4	10-4	□	□	-	-
5	10-5	-	-	-	-

Table 2 shows that E. coli and Salmonella bacteria were grown on both selective growing media. Bacteria are present in dilutions of 10-1 to 10-4. In EMBA media, there were E. coli

bacteria at a dilution of 10-1 to 10-4. This is in accordance with what Masduki (1996) said, that the bacteria that grow in EMBA media are only E. coli bacteria, while other bacteria cannot live. Meanwhile, in SSA media, the bacteria that grew in the 10-1 and 10-2 dilutions were not Salmonella but E. coli.

It is known that coliform bacteria are bacteria that live in the human digestive tract. Where one member of the coliform group according to Masduki (1996) is E. coli present in human feces. However, it can be found in the tested compost. This shows that the compost used is contaminated with human waste.

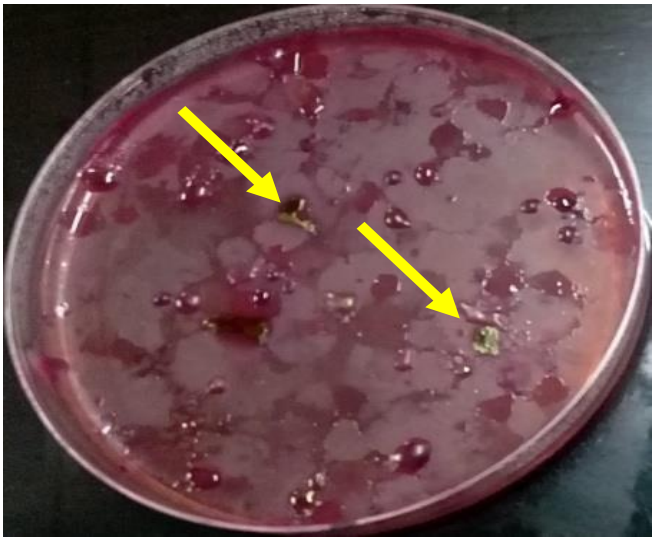

Image 1 There is E. coli bacteria on the EMBA media, it looks metallic green

3. Sample III (Lettuce Leaves)

Table 3 Identification of E. coli and Salmonella bacteria in sample III (lettuce leaves) with multiple dilutions of 1 his

No	Dilution	Test			
		EMBA		SSA	
		1	2	1	2
1	10-1	□	□	□	□
2	10-2	□	□	E. coli	+ E. coli
3	10-3	□	□	E. coli	-
4	10-4	□	□	-	-

Table 4 shows that E. coli and Salmonella bacteria were grown on both selective growing media. Bacteria are present in dilutions of 10-1 to 10-4. In EMBA media, there were E. coli bacteria at dilutions 10-1 to 10-4 in replicates 1 and 2. While in SSA media, bacteria that grew in dilution 10-1 were Salmonella bacteria, while in dilutions 10-2 and 10 -3 even E. coli bacteria.

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This is as Anonimus (2008) said, where the main sources of bacterial infection are raw food, undercooked food and cross-contamination, namely when cooked food comes into contact with raw materials or contaminated equipment.

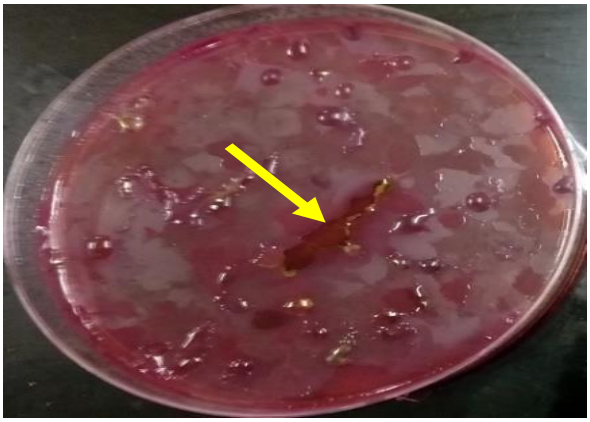


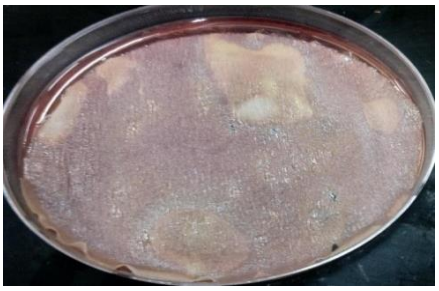
Figure 2 There is E. coli bacteria on the EMBA media, it looks metallic green

4. Sample IV (Water)

Table 4 Identification of E. coli and Salmonella bacteria in IV samples (well water and refill water) with multiple dilutions of 1 his

No	Sample	EMBA		SSA	
		Test		Test	
		1	2	1	2
1	Well	□	*Salmonella*	-	-
2	Refillable	Other bacteria	Other bacteria	*Shigela*	*Shigela*
3	Well (filter paper)	□	-	□	-
4	Refill (filter paper)	□	-	□	-

Table 4 shows that E. coli and Salmonella bacteria were grown on both selective growing media. In EMBA media, E. coli bacteria were found in well water for replication 1, filter paper well water and filter paper refills. While on SSA media, there were Salmonella bacteria in well water samples (filter paper) and refills (filter paper). However, apart from that, there were also other bacteria, namely Shigella, on AAS media in the refill sample.



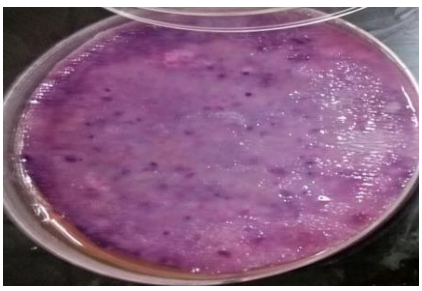


Figure 3 There are E. coli, Salmonella and Shigella bacteria on EMBA and SSA . media

3.2 Bacterial Colony Number

The results of observing the number of colonies of E. coli and Salmonella bacteria in the three samples in EMBA and SSA media 2 days after inoculation are in Table 5:

Table 5 Number of colonies of E. coli and Salmonella bacteria on EMBA and SSA media media

No	EMBA Media (Colonies)	SSA Media (Colonies)
1.	2 x 10-3 (repeat 2) = 0	3 x 10-2 (repeat 1) = 27
2.	2 x 10-2 (repeat 2) = 0	3 x 10-2 (repeat 2) = 39
3.	3 x 10-1 (repeat 1) = 79	2 x 10-1 (repeat 1) = 3
4.	2 x 10-1 (repeat 2) = 3	2 x 10-1 (repeat 2) = 2
5.	3 x 10-1 (repeat 2) = 59	3 x 10-4 (repeat 1) = 0
6.	2 x 10-1 (repeat 1) = 14	2 x 10-2 (repeat 1) = 0
7.	2 x 10-3 (repeat 1) = 0	2 x 10-2 (repeat 2) = 0

The highest number of colonies of E. coli in EMBA media was found in sample 3 (compost fertilizer) with 1 replications 1 and 2, which were 79 and 59. Followed by sample 2 (lettuce) with 1 replications 1 and 2, which were 14 and 3. dilutions 2 and 3 did not find these bacteria. Likewise, the highest number of Salmonella bacterial colonies in SSA media was found in sample 3 (compost fertilizer) with dilutions of 2 replicates 1 and 2, namely 27 and 39. Followed by sample 2 (lettuce) with 1 replications 1 and 2 dilutions of 3 and 2, while In dilution 2, no Salmonella bacteria were found.

3.3 Confectionery Fermentation Test

Identification of E. coli and Salmonella bacteria can also be tested using the confectionery test. (glucose, sucrose and lactose). Identification data can be seen in Table 6:

Table 6 Test of sugars (glucose, sucrose and lactose) against E. coli and Salmonella bacteria

Pathogenic bacteria	Fermentation occurs by producing gas/bubble water		
	Glucose	Sucrose	Lactose
E. coli	□	□	-
Salmonella	□	□	-

The yellow color on the whole media was caused by E. coli in TSIA media that can ferment glucose, lactose and sucrose. Positive gas because the gas produced by carbohydrate fermentation

will appear as a gap in the media or will lift the agar from the bottom of the tube (Hadioetomo, 1993).

Table 7 Fermentation test (glucose, sucrose and lactose) against E. coli and Salmonella bacteria

Pathogenic bacteria	Fermentation test		
	Glucose	Sucrose	Lactose
E. coli	+++	+++	+
Salmonella	+++	+++	+

Information :
+ : can ferment sugar in small amounts
++ : can ferment sugar in moderation
+++ : can ferment sugar in large quantities

The results of the observations for the biochemical test of sugars were positive except for mannitol. The color of the media changes to yellow, it means that the bacteria form acid from glucose fermentation (the phenol red indicator in acidic pH turns yellow). Any carbohydrate can be used, but the most commonly used are glucose, lactose, and sucrose. The yellow color in the entire medium was due to the fact that E. coli in TSIA media can ferment glucose, lactose and sucrose (Susan, 2006).

Durham tubes are placed upside down in each tube as an indicator of gas production. Gas production is indicated by the presence of bubbles in the Durham tube. This is as stated by Leboffe (2011), where the ability of this medium to detect acid production depends on the incubation time and the fermenter's ability to produce an excess of acid relative to the ammonia produced by the deamination process. Positive gas because the gas produced by carbohydrate fermentation will appear as a gap in the media or will lift the agar from the bottom of the tube.

4. CONCLUSION

From the results of this study, several conclusions can be drawn, namely:

1. The samples of cocoa powder, EMBA and SSA media did not contain E. coli and Salmonella sp.
2. In the compost sample, the EMBA media contained E. Coli bacteria while the SSA media did not grow Salmonella sp.
3. In lettuce samples, in EMBA media there were E. coli bacteria while in SSA media there were Salmonella and E. coli.
4. In water samples, EMBA media contained E. coli bacteria, Salmonella sp. and other bacteria while the SSA media contained Salmonella sp. and Shigella.
5. In the fermentation test using sugars such as glucose, sucrose and lactose, the media changes color to yellow and there is gas/air bubbles, which means bacteria can ferment carbohydrates and can produce gas.

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