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Identification of Bacteria in Fresh Vegetables from The Traditional Market of Mataram City

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Abstract: Indonesia is very rich in potential for vegetable cultivation. Vegetables contain a lot of vitamins and minerals. Therefore, people prefer to eat vegetables in a fresh state either as salads or fresh vegetables. However, contamination of pathogenic bacteria is much greater both at the farmer and trader levels. Therefore this study aims to determine the feasibility of consumption and identify bacteria in fresh vegetables in the traditional market of Mataram City. Samples in the form of lettuce, cucumber, cabbage, and long beans were taken from Pagesangan, Kebon Roek, Gunung Sari, and Bertais markets, respectively. The existence of bacteria were detected using traditional method by inoculating samples in agar cultures followed by biochemical testing and Gram staining. Species of bacteria that can be identified from samples are *Enterobacter cloacae*, *Bhanhamella catarrhalis*, *E-coli*, *Staphylococus sapropyticus*, and *Aeromonas hydrophila*. Based on the data, long beans from the Bertais market and lettuce from the Pagesangan, Kebon Roek, and Gunung Sari markets are not suitable for consumption because the TPC yield exceeds the consumption qual**ity** standard of 105-106 CFU/g.

Keywords: bacteria pathogen; TPC; vegetable quality standards

Introduction

Natural conditions in Indonesia make it possible to cultivate various types of vegetables, both locally and from abroad. In terms of agro-climatological aspects, Indonesia has great potential for vegetable cultivation. In addition, technical, economic, and social aspects also support vegetable entrepreneurs in Indonesia (Siahaan, 2010). According to data from the 2009 Central Bureau of Statistics, the average daily calorie consumption for these vegetables has increased, namely in 2002 by 37.44 cal and in 2007 it increased to 46.39 cal (Siahaan, 2010). This can indicate that people's awareness to consume fresh fruits and vegetables is getting higher.

A healthy lifestyle is currently in demand by the public. One form of a healthy lifestyle that is applied today is a pattern of consuming fresh vegetables (Setiawan, 2020). Vegetables and fruits contain lots of vitamins and minerals that are needed by the body. The content of vitamins and minerals will decrease if the vegetables and fruits are processed. Therefore, nowadays many people prefer to eat fresh vegetables and fruits.Lalapan is also often consumed in the form of salads in European and American countries. People in Indonesia often consume fresh, unprocessed vegetables known as fresh vegetables, including cucumbers, basil, lettuce, long beans, cabbage, and tomatoes (Suryani, 2012)

The nutritional content of fresh vegetables is better than cooked vegetables, but the risk of contamination by pathogenic bacteria is much greater (Metisya, 2016). This can occur due to poor treatment of fresh vegetables at the farmer level to the trader level so contamination often occurs during harvesting, transportation, or marketing. Bacterial infection is a zoonotic infection and is known as salmonellosis. In the

human body, salmonellosis caused by S. typhi causes a fairly high increase in body temperature, or is known as typhoid fever. Typhoid fever is known to be a cause of high morbidity and mortality in various countries, especially developing countries, including Indonesia (Idar et al., 2018). Some foodstuffs that are not cooked perfectly are also a common medium for transmitting these bacteria. Examples of foodstuffs that are often occupied by Salmonella are meat, eggs, dairy products, fruits, and vegetables (Barakat, 2011). Vegetables generally contain relatively high levels of carbohydrates with a pH of 5.0 – 7.0. This makes vegetables a good medium for the growth of many types of bacteria, yeasts and molds when conditions are suitable. Microorganisms in vegetables can come from the soil, water, air, livestock including poultry, insects, birds, or equipment that varies depending on the type of vegetables.

Vegetables generally contain relatively high levels of carbohydrates with a pH of 5.0 – 7.0. This makes vegetables a good medium for the growth of many types of bacteria, yeasts and molds when conditions are suitable. Microorganisms in vegetables can come from the soil, water, air, livestock including poultry, insects, birds, or equipment that varies depending on the type of vegetables (Kustyawati, 2020). The number and types of microbes in vegetables also vary, depending on environmental conditions, agriculture, and harvest conditions. The dominant bacteria in vegetables are *lactic acid, Corynebacterium, Enterobacter, Proteus, Pseudomonas, micrococcus, Enterococcus,* and spore-forming bacteria (Sopandi, 2014).

Pathogenic bacteria that can contaminate vegetables *Staphyloccocus aureus, Salmonella spp.,* and *Listeria monocytogenes*, are often associated with food derived from fresh products including vegetables (Primaharani, 2021). Therefore, consuming raw vegetables can cause foodborne diseases. Foodborne Disease is a disease that is the result of digestion and absorption of food that has been contaminated with microbes or agents in the human body. Based on the Indonesian Ministry of Health in 2018, the highest food poisoning is the result of food that has been contaminated by pathogenic bacteria (Kementerian Kesehatan Republik Indonesia, 2018). Therefore, researchers are interested in identifying bacteria in fresh vegetables in the traditional market of Mataram City.

Material and Methods

Time and place of research

This research is an experimental study with a descriptive qualitative approach. The research was conducted in October-November 2021 at the Testing and Calibration Laboratory of West Nusa Tenggara Province.

Instruments

This research used petri dishes**,** test tubes, tuberacks, scissors, tweezers, ose, Bunsen, scales, pipettes, vortex, Erlenmeyer flasks, incubators, autoclaves, water heaters, glass spatulas, sterile, refrigerators, lighters, camera, marker, label, sterile plastic clip, plastic wrap, tissue, stirrer.

Materials

Infusion), physiological salt, XLD (Xylose, Lysine, Deoxycholate) media, nutrient agar (NA), gentian violet, safranin solution, 96% alcohol, Lugol's solution, immersion oil, oxidase strip, H202 10% (Hydrogen Peroxide 10%), glucose, lactose, mannitol, maltose, simoncitrater, TSI (Triple Sugar Iron), SIM (Sulfi, Indole, Motility), AP (Water Peptone), GP (Glucose Phosphate), ureas.

Methods

Sterilization of tools and materials

After the tools and materials are prepared, then all the tools to be used are washed and then dried and wrapped in corn paper, and then sterilized in an autoclave for 15 minutes at 121°C.

Sampling

Samples of fresh vegetables such as lettuce, cabbage, cucumber, and long beans at the traditional market of Mataram city were taken and then put into sterile plastic clips.

Testing Stage

Pre-enrichment Sample in a sterile plastic clip is taken by cutting it into small pieces using a sterile knife and weighing 10 grams, then putting 90 ml of distilled water into a sterile

plastic clip that already contains the vegetables, then crushed.

Isolation and identification

Then, 100 microliters of the vegetable sample was taken using a sterile pipette and put into physiological gram media (10-1-10-4) for dilution. Next, 100 microliters of vegetable dilution was taken using a sterile pipette, then inoculated on XLD media using the pour plate method. Then incubated at a temperature of 35°C for 24 hours ± 2 hours. After that, observe the colonies of pathogenic bacteria that grow on XLD media. Colonies formed on XLD media were clear yellowish in color and round in shape.

Bacteria purification

The colonies contained in the XLD media are yellow in color and are round in shape and grow separately, then purified by taking 1 ose, then streaking on slanted NA media (Nutrient Agar) as stock for gram staining and biochemical tests. Then incubated at 35 °C for 24 hours.

Biochemical

Tests Biochemical tests were carried out using several ingredients, namely glucose, lactose, mannitol, maltose, simon citrate, TSIA, SIM, GP/ Methyl Red, AP/Indol, Urea, Catalase, and Oxidase. The TSIA test was used for gram-negative bacteria that ferment glucose, lactose or sucrose, and also forms hydrogen sulfide. TSIA contains *phenol red* which functions as an acidity indicator and *ferrous sulfate* which functions as a H2S formation large amount of lactose and sucrose fermentation occurs in the agar sloping part while at the bottom of the agar, glucose fermentation occurs so that the color changes to yellow.

Gram staining

The slide is preheated over a Bunsen flame for sterilization. After that, take one drop of sterile distilled water using a dropper. Then the slide is marked with a marker to mark the place to put the colony. Take the colonies from the SSA media with the ose and then flatten them on the slide. Fixation of the preparation by passing it over the fire as much as 8-10 times and cooling the preparation at room temperature.

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The first thing to do is drip the preparation with gentian violet solution on gram staining, then let it stand for 3 minutes. Next, rinse with running water and add lugol drops and let stand for 1 minute. When returning with running water and drops of 96% alcohol, then rinse with clean running water. Drops of safranin let stand for 45-60 seconds then rinse with running water. After that dry with a tissue. Then drop 1 drop of immersion oil and look at the microscope with 100x magnification. The results obtained are pathogenic bacteria with gram-negative characteristics marked with red color.

Results and Discussion

Bacterial isolation

The first step is to isolate bacteria from samples of fresh vegetables, namely lettuce, long beans, cabbage, and cucumber. Each sample was taken from four traditional markets in Mataram City, namely Pagesangan Market, Kebon Roek, Bertais, and Gunung Sari. At this stage, the process equipment was incubated for 15 minutes at 121°C. The goal is to sterilize the tool so that it is not contaminated with outside bacteria. Next is the sample cutting process. Samples were cut into small pieces and soaked with distilled water.

The next process is dilution using the pour plate method or the scatter method. This means that the collection of cells that have been diluted will be evenly distributed throughout the dish, not just on the surface. Dilution is done in decimal in four stages, namely 10-1, 10-2, 10-3, and 10-4. Then incubated at a temperature of 35°C for 24 hours ± 2 hours. The goal is to see the growth of bacteria at low temperatures so that the bacteria can survive until they can be isolated. The colonies observed were clear yellowish in color and round in shape.

Calculation of Total Plate Count (TPC)

The number of bacterial colonies from the sample is calculated using the formula:

$$Koloni/gr = \Sigma\ koloni\ per\ cawan\ x \frac{1}{faktor\ pengenceran}$$

The calculation of the TPC or the number of bacterial colonies in the dish is carried out by observing the standard TPC calculation. First, the selected and counted dishes were those containing the number of colonies between 30-300 CFU/g. If the number of colonies per sample is more than 300 CFU/g, it is categorized as too many to count (TBUD) or too numerous to count (TNTC) (Soesetyaningsih, & Azizah. 2020). Second, several colonies that merge into one are a large collection of colonies where the number of colonies can be counted as one colony.

Third, the colony chain as a thick line is counted as one colony. Fourth, the data reported only consists of two numbers, namely the first number in front of the comma and the second number after the comma. If all the dilutions made produce the number of colonies below 30, then only the number of colonies at the lowest dilution is counted. If all the dilutions made resulted in the number of colonies above 300, then only the number of colonies at the highest dilution was counted (Kustyawati, M. E. (2020).

Based on the above standards, the results of the TPC calculation for the four samples can be seen in table 1.

Table 1. TPC calculation results

Market Name	Total Plate Count (CFU/g)			
	Cucumber	**Long beans**	**Cabbage**	**Lettuce**
Pagesangan	0,5 x 10^5	5,2 x 10^5	1,9 x 10^5	30,1 x 10^5
Kebon Roek	0,5 x 10^5	6 x 10^5	1,6 x 10^5	30,1 x 10^5
Gunung Sari	0,2 x 10^5	2,3 x 10^5	4,1 x 10^5	10,3 x 10^5
Bertais	0,3 x 10^5	16,7 x 10^5	0,3 x 10^5	2,6 x 10^5

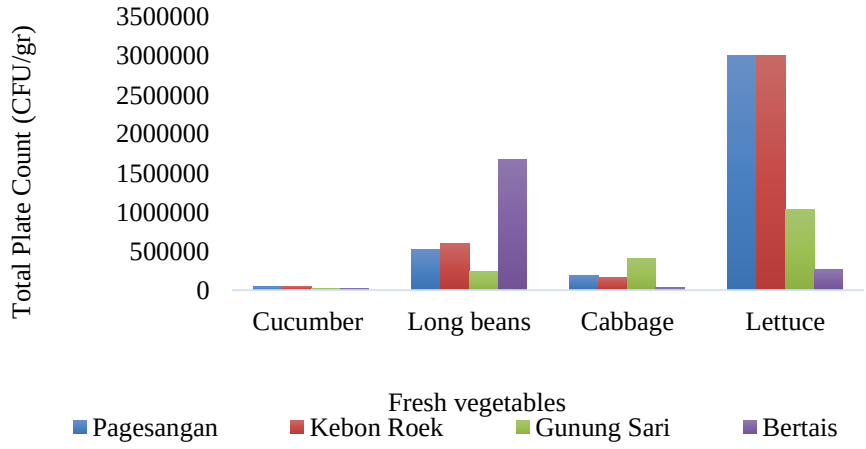

Figure 1. Graph of TPC calculation

The calculation results obtained very many bacterial colonies, especially lettuce in the Pagesangan and Kebon Roek markets, exceeding 300 CFU/g. The purpose of this TPC calculation is to determine the feasibility of consumption by taking into account the microbiological quality standards of fresh vegetables. Based on the International Commission on Microbiological Specification for Foods (ICMSF) 1996 the standard TPC (Total Plate Count) of vegetables to be eaten raw is n = 5, c = 3, m = 105, and M = 106, meaning a maximum of 3 samples from the 5 samples analyzed may contain a total of 105106 CFU/g microbes (Siahaan, 2010). The 2009 National Standardization Agency (BSN) it was also stated that the bacterial standard in fresh vegetables was 105CFU/g (BSN. 2009).

The results of the study found that vegetables that did not meet quality standards at Pagesangan Market, Kebon Roek, and Gunung

Sari were lettuce, which was more than 106 CFU/g. Meanwhile, at the Bertais Market, vegetables that did not meet quality standards were long beans at more than 106 CFU/g. This is influenced by spraying or irrigating with rice field water and fertilizing with animal manure so that in vegetables such as lettuce and long beans, many bacterial colonies are found so that they do not meet quality standards suitable for consumption. As seen from the graph in Figure 1, the number of bacterial colonies on lettuce in Pagesangan and Kebon Roek markets is very high compared to the other two markets.

Bacterial contamination of vegetables occurs at several points from production, harvest, processing, and distribution to marketing stages (Idar, et. al., 2018). The above statement is in accordance with the results of interviews with traders in the market where they take vegetables from farmers who were previously washed with rice fields so that bacterial contamination increases. This is also reinforced by the condition of laying vegetables in the market. Vegetables are only placed on a table without a base or spread out on the ground with a plastic or bamboo basket.

Vegetable contamination by microorganisms can be caused by vegetable farmers who use feces a lot as fertilizer which is likely to contain pathogenic bacteria, viruses, or parasites. This of course will not be a problem if the vegetables are not eaten raw or washed properly before consumption (Iqbal, 2019). The use of phytochemical compounds such as phenolic compounds from plants can be used as an alternative to synthetic disinfectants because these compounds can be used as antibacterials. One of the natural ingredients that can be used as a natural disinfectant in fresh vegetables is petai skin (Azhar, 2020). In addition, the manufacture of betel leaf antiseptic can also be used to wash fruits and vegetables to inhibit the growth of Salmonella sp bacteria as the cause of typhoid (Silvian & Saktiningsih, 2020).

Gram staining and biochemical test

Gram stain and biochemical tests were carried out only in two markets, namely Pagesangan and Kebon Roek. Before the biochemical test was carried out, gram staining was carried out first. The aim was to identify the type of bacteria based on their physical characteristics in the form of morphology (*coccus*, *bacillus*, and *spiral*) and their arrangement (chains, groups, pairs, and tetrads) as well as internal structures of bacteria such as cell walls and vacuoles. The results can be seen in table 2 and table 3 below.

Table 2. Table of biochemical tests and gram staining in Kebon Roek

Biochemical Test	**Vegetables on the market Kebon Roek**			
	Lettuce	**Long beans**	**Cabbage**	**Cucumber**
Gram	-	-	-	-
Glucose	+	+	+	+
Lactose	+	+	+	+
Mannitol	+	+	+	+
Maltose	+	+	+	+
Simon Citrat	-	-	-	-
TSI	-	-	-	-
Sulfi	-	-	-	-
Indol	+	+	+	+
Motility	+	+	+	+
GP	+	-	-	-
AP/Indol	+	+	+	-
Urea	+	-	+	-
Catalase	+	+	+	+
Oxidase	-	-	+	-
Bacteria Type	*E-coli Bacil*	*Aeromonas hydrophila Diplococus*	*Bhanhamella catarrhalis Coccus*	*Enterobacter cloacae Cocobasil*

Table 3. Biochemical Test and Gram Staining Table Pagesangan

Biochemical Test	Vegetables on the market Pagesangan			
	Lettuce	**Long beans**	**Cabbage**	**Cucumber**
Gram	-	-	+	-
Glucose	+	+	+	+
Lactose	+	+	+	+
Mannitol	+	+	+	+
Maltose	+	+	+	+
Simon Citrat	-	-	-	-
TSI	-	-	-	-
Sulfi	-	+	-	-
Indol	+	+	+	+
Motility	+	+	+	+
GP	-	-	-	-
AP/Indol	+	+	+	-
Urea	+	+	+	-
Catalase	+	+	+	+
Oxidase	+	+	+	-
Bacteria Type	*E-coli Bacil*	*Bhanhamella catarrhalis Cocobasil*	*Staphylococus saprophyticus Coccus*	*Enterobacter cloacae Cocobasil*

The results of the n analysis obtained on all samples at the Kebon Roek market found gram negative except for cabbage. This means that the bacteria in cabbage are gram-positive, where the bacteria have a thick cell wall structure of 15-80 mm, single-layered or monolayer. On the other hand, for gram-negative cells, the cell wall structure is thin, which is 10-15 mm. The forms of bacteria that were identified

Were *coccus* (round), *diplococcus, bacillus* (rod), and *cocobasil*. Biochemical tests are carried out to identify the types of bacteria based on their chemical characteristics. Based on the data analysis, the types of bacteria in the Pagesangan Market were *Enterobacter cloacae* on cucumber, *Bhanhamella catarrhalis* on long beans, *E-coli* on lettuce, and *Staphylococus sapropyticus* on cabbage. Furthermore, Pasar Kebon Roek found *Enterobacter cloacae* on cucumbers, *Aeromonas hydrophila* on long beans, *E-coli bacteria* on lettuce, and *Bhanhamella catarrhalis* on cabbage.

Enterobacter cloacae is a pathogen that causes nosocomial infections and is responsible for various infections including, respiratory tract infections, urinary tract infections, sepsis, intra-abdominal infections, skin and soft tissue infections, eye infections, and gastrointestinal infections (Riga, P. N., et al. 2015). One of the diseases caused by *Moraxella catarrhalis* (*Bhanhamella catarrhalis*) is otitis media (inflammation of the middle ear). Divided into acute otitis media, otitis media with effusion, and chronic otitis media. This infection is common in children. Acute otitis media is characterized by local inflammation, otalgia, otorrhea, irritability, lack of rest, decreased appetite, and fever. Acute otitis media can cause pain, hearing loss, fever, leukocytosis (Bandangan, 2016).

The most common diseases caused by *Escherichia coli bacteria* are urinary tract infections, prostatitis, and pelvic inflammatory disease (PID). Escherichia *coli bacteria* usually inhabit the digestive tract causing diarrhea (Hutasoit, 2020). While *Staphylococcus aureus* is a normal flora on human skin, but under conditions that allow it to infect human skin, it can cause acne and boils. *Staphylococcus aureus* can also infect wounds, then enter the bloodstream to spread to other organs and cause pneumonia, infections of the heart valves that trigger heart failure, bone inflammation, and can even cause shock that can cause death (Huda, 2013). Furthermore, *Aeromonas hydrophila* causes Motile Aeromonas Septiemia (MAS) disease which attacks several internal organs such as the liver, spleen, and kidneys (Muslikha, et al. (2016).

Conclusion

Based on the description above, it can be concluded that the number of bacterial colonies

on lettuce in Pagesangan Market, Kebon Roek, Gunung Sari, and long beans in Bertais Market exceeded 106 CFU/g. Both of these vegetables do not meet the quality standards of vegetables fit for consumption. The types of bacteria identified in fresh vegetables at the Pagesangan and Kebon Roek Markets were *Enterobacter cloacae*, *Bhanhamella catarrhalis*, *E-coli*, *Staphylococus sapropyticus*, and *Aeromonas hydrophila.*

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