Identification of Vibrio in green clam

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Identification of Vibrio sp. in Green Clam (Perna viridis) at West Bekasi Traditional Market

Indonesia is an archipelagic country with the potential for an abundant wealth of marine products such as various types of fish, shellfish and others. Communities who live far from coastal areas can obtain fishery products that are sold in the market. Market conditions could be better organized, clean enough and have better environmental sanitation. This condition can cause other organisms to multiply, such as bacteria. Green clam (Perna viridis) is one of several types of shellfish widely consumed by the public. Green clam (Perna viridis) contains much protein. However, the processing of the mussels could be better, and they could be consumed without going through the cooking process. In that case, it can be a source of transmission and contamination from microorganisms and chemicals in the waters. This study aims to obtain information on the cleanliness and safety of green mussels, foods often eaten by the community. The method used is the spread plate method, Gram staining, and biochemical tests. Sampling was done by grinding raw green mussels and then adding sterile aquadest with a ratio of 1:1. 0.1 mL of water was taken and placed in TCBS media, then spread with drugalski, then incubated for 24 hours at 37 degrees. The growth of colonies on TCBS selective media concluded that they were Vibrio sp. colonies. It was also found that gram-negative bacteria in the form of bent bacilli, like commas on gram staining, were the strongest reference for identifying Vibrio sp. In conclusion, in this study, four green clams from traditional markets in the Kranji area, West Bekasi, were used to contain Vibrio sp. bacteria caused by faecal contamination containing these bacteria in seawater. Communities must be more careful in choosing green clams to eat with their families, make sure the seller's stalls are clean, clean the shells properly, and process the shells by applicable recommendations.

Keywords: Green clam, Traditional market, Vibrio sp.

According to Yuhantaka (2018), Indonesia is an archipelagic country with the potential for an abundant wealth of marine products, such as various types of fish, shellfish, and others. Communities who live far from coastal areas can obtain fishery products that are sold in the market. In general, market conditions could be better organized, cleaner, and better environmental sanitation. This condition can cause other organisms to multiply, such as bacteria. Green clam (Perna viridis) is one of several types of shellfish widely consumed by the public. Green clam (Perna viridis) contains much protein. However, the processing of the mussels could be better, and they could be consumed without going through the cooking process. In that case, it can be a source of transmission and contamination from microorganisms and chemicals in the waters. Shellfish live in water, so they absorb seawater and nutrients that settle on the seabed to clean seawater so that materials such as heavy metals can also be absorbed into their bodies. If seen from the process of shellfish filtering water to get food, shellfish are vulnerable to contamination by harmful microorganisms. Dangerous microorganisms, such as pathogenic bacteria in food, can trigger foodborne disease (Devi et al., 2019). According to the Regulation of the Head of the Food and Drug Supervisory Agency (BPOM, 2009) RI No.HK.00.06.1.52.4011 of 2009, which confirms the limit in consuming seafood for fishery product types contaminated with Vibrio sp. bacteria to reduce the occurrence of Foodborne disease, namely negative every 25 grams (Suliyaningsih, 2020). According to Hikmawati,

Susilowati, and Ratna (2019), Foodborne disease is a disease caused by consuming food that has been contaminated with bacteria. The incidence of foodborne disease is caused by consuming seafood. As much as 10-20% of cases are caused by the bacterium Vibrio sp. Vibrio sp. are pathogenic or harmful bacteria for humans that will cause gastroenteritis. Gastroenteritis is the increase and decrease in the frequency of stool consistency compared to the subject with colon disease. Gastroenteritis is loose stools (feces), liquid / semi-liquid (semi-solid), and much water. This lasts less than seven days and occurs spontaneously. The main causes of acute gastroenteritis are bacteria, viruses, Helicobacter pylori, and Vibrio cholerae. Other parasites can also cause gastritis. Symptoms that often arise are vomiting, abdominal pain, stomach cramps, and frequent diarrhea, so sufferers will lose a lot of fluids and electrolytes, which will cause severe dehydration (Suliyaningsih et al., 2020). Meanwhile, according to SNI No.7388:2009, clams cannot contain Vibrio sp. If this happens, it indicates that the shellfish has been contaminated and is not good for consumption because it can cause foodborne diseases such as diarrhea (Annisa, 2017). Vibrio sp. has characteristics including a short rod shape, is a Gram-negative bacteria, has a flagellum, does not have spores, does not have a capsule, is facultatively aerobic and reproduces by binary fission, grows on Thiosulfate Citrate Bile-salt Sucrose Agar medium (Muna, 2021). Kranji Market is an area near where we live. Several times, we bought food ingredients (including green clams) there. So, for that reason, we finally chose Kranji market as the place where we took samples of green clams, which were suspected to contain Vibrio sp. bacteria.

The research method used is descriptive observation. Data were collected from the West Bekasi Traditional Market on June 8, 2023. The results of the data were obtained after practical learning. Green clam samples were extracted by crushing 2 grams and giving them distilled water in a ratio of 1:1, then 0.1 mL of distilled water was taken and spread on TCBS media.

1

The research results on green mussels or Perna viridis in TCBS media were yellow colonies with small round shapes and convex elevations. The results for colonies on MCA are pink colonies with a rounded shape, convex elevation, and wavy edges. Gram staining shows the shape of bent rod bacteria like commas with red color, which indicates Gram-negative bacteria according to the literature. The MR and VP results show positive MR results, as seen from the formation of a red color change after dropping the Methyl Red reagent and negative VP because the media does not change color. It remains yellow. The SIM test showed positive sulfite and motile results following the literature showing that Vibrio sp. has a flagellum to move. In contrast, the indole test was not carried out due to the unavailability of indole reagents. The TSIA test showed negative slant and positive butt results, as seen from the change in the color of the media, which was previously red to yellow. The SCA test shows a negative result because the media remains green. The results of the sugar test showed that the results for glucose, mannitol, and maltose were negative. The results for lactose and sucrose were positive because the media changed color from yellow to orange. The last one is the catalase test. The catalase test showed a positive result because bubbles formed after dropping H 2 O 2 . TCBS media (Thiosulphate-Citrate-Bile Salts-Sucrose), spread with green clam water and incubated for approximately 24 hours at 37°C, resulted in a single colony

growing yellowish. According to research conducted by Hikmawati, Susilowati, and Setyaningsih (2019) states that the yellow colonies on TCBS media are Vibrio sp. bacteria. Green clams are used as samples in practicum because Vibrio sp. is often found in seawater and seafood, especially green clams. Vibrio sp. can give a yellow color to TCBS media because Vibrio sp. ferments sucrose in the media, so a yellow color is formed. TCBS media is a selective and differential medium for the growth of Vibrio sp. bacteria because it contains bile salts, NaCl, thymol blue, ox bile, agar, sucrose, bromothymol-blue, yeast extract, sodium trisulfate, bile-salts function to inhibit bacterial growth besides Vibrio sp. NaCl is the optimal medium for halophilic growth, and sodium trisulfate, a source of sulfur and ferric citrate, is used to detect H2S production (Muna, 2021). Vibrio sp. obtained from a sample of green clam gave a red color with a bent rod shape (like a comma) on Gram staining, classified as a Gram-negative rod-shaped bacteria. Gram stain has two types, namely Gram-positive and Gram-negative. Cappuccino and Sherman (2013) suggested that Gram staining was carried out to distinguish between Gram-positive and Gram-negative bacteria. Gram-negative bacteria do not have a thick peptidoglycan, so the primary dye (crystal violet) will fade when given a bleach (Lugol's iodine), and the bacteria will be stained red by the secondary dye (safranin). The MR (Methyl-Red) test carried out on samples of green mussels gave the result of a change in the color of the media to red after being given the Methyl-Red reagent so that it was identified as positive in the MR test. These results are to the statements of Suliyaningsih, Arifin, and Ismunanti (2020) through their research, which stated that the MR test was carried out to determine the ability of bacteria to oxidize glucose and produce acid as the final product so that the media will turn red. The VP test (Voges-Proskauer) on green clam samples suspected of containing Vibrio sp. gave negative results, which were indicated by the absence of a color change in the medium from yellow to reddish after adding alpha-naphthol and KOH reagents. This is by the statement of Suliyaningsih, Arifin, and Ismunanti (2020), which stated that a negative result on the VP test was marked by no change in the color of the media to reddish because the VP test was carried out to determine the ability of bacteria to produce acetyl methyl carbinol from organic acids in glucose metabolism. Furthermore, Vibrio sp. does not produce diacetyl or acetoin. Vibrio sp. that had been grown on SIM (Sulfide Indole Motility) media gave positive indole and motility results but negative sulfide, which was indicated by the presence of a blackish-red ring on the surface of the media and visible branching colonies from the loop needle puncture marks, but no black precipitate on the surface of the media. Media, after incubation, for approximately 24 hours. Cappuccino and Sherman (2013) stated that SIM media contains peptone and sodium thiosulfate as sulfur substrates, FeSO 4 acts as an H2S or sulfide indicator so that there is no reaction between FeSO 4 and H 2 S, and the media is not blackish. The reaction between p-dimethylaminobenzaldehyde, butanol, and hydrochloric acid by Vibrio sp. produces a red-black layer/color. It shows movement with the growth spread from puncture marks because Vibrio sp. is motile. The SCA (Simmon's Citrate Agar) media test on the green clam sample showed no change in the media color to blue after being incubated for approximately 24 hours at 37°C, so it was indicated as negative. This can happen because the sodium carbonate in the SCA medium does not change the bromothymol-blue indicator in the media. After all, it does not ferment citrate into a carbon source to precipitate energy due to the unavailability of glucose (Cappuccino & Sherman, 2013). The sugar test that was carried out gave positive results on the sucrose and lactose tests but negative on the glucose, mannitol, and maltose tests, which were indicated by a change in color from orange to yellow on mannitol, maltose, and lactose media and no color change

on the media. Glucose and sucrose (stay orange). This shows that Vibrio sp. cannot ferment glucose and sucrose but can ferment lactose, maltose, and mannitol (Cappuccino & Sherman, 2013). Vibrio sp. from a sample of green clam grown on MCA (Mac Conkey Agar) media gave purple colonies, and there were other yellow colonies. Vibrio sp. produces purple colonies because Vibrio sp. can ferment lactose on MCA media and grows well because MCA media is a differential selective medium for Gram-negative bacteria, so Vibrio sp., a Gram-negative bacteria, can grow well. However, yellowish colonies are thought to be Gram-negative bacteria that do not ferment lactose because these bacteria cannot lower the pH of the media, which is detected by a neutral red indicator due to lactose fermentation (Cappuccino & Sherman, 2013).

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