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Analysis of Pathogenic Bacteria in Oysters (*Crassostrea cucullata)*

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Abstract: The research objective was to analyze the pathogenic bacteria in oysters (*Crassostrea cucullata*). This research was conducted at the Makassar Laboratory (BBLK). The samples used were oyster meat and innards. Parameters observed were Escherichia coli and Salmonella sp. Research on samples was carried out using biochemical tests to identify the type of bacteria in the sample by planting the bacteria on Nutrient Agar (NA) media. The results showed that oyster meat in Lengkese Village, Takalar Regency was negative for E. coli and Salmonella sp. While the oyster meat samples in Coppo Village, Barru Regency showed positive E. coli bacteria and negative Salmonella sp.

Keywords: *Escherichia coli*; Oysters; *Salmonella sp.*

Introduction

Seafood that contains high-quality protein, omega-3 fatty acids, and a variety of vitamins and minerals. Many Indonesians consume seafood as their primary source of EPA and DHA. The high consumption of seafood in Indonesia is due to the fact that the country is surrounded by water, so most people eat seafood as their main side dish (Mukhtasor et al., 2021).

In general, pathogenic bacteria can grow well on media in the form of meat such as oyster meat and are the main trigger for causing food poisoning. According to the Food and Agriculture Organization (FAO) more than 80% of food poisoning is caused by pathogenic bacteria, including staplycoccus, salmonella, and clotrodium betalicum. Cl Perferigersus and Bacillus eurus.

Oyster (Crassostrea cucullata) is a type of bivalves that is commonly consumed by coastal communities. This species lives in estuaries attached to mangrove roots, piers, and various dead rock objects at the bottom of the water. The country that has intensively cultivated this type of oyster is the Philippines (Maulidza et al., 2019).

Oysters live in estuaries attached to mangrove roots, piers, and various stone objects, as well as dead coral on the seafloor. The upper shell is smaller than the lower shell and has an irregular shell shape that is asymmetrical with a small hump (Putri et al., 2021).

Oysters are divided into three parts: the legs, the shell, and the inner flesh. During the larval season, the legs are used as limbs to move or crawl before settling/attaching to the substrate. These organisms, however, use cement to glue themselves together once they reach maturity. That's why his legs don't work. The mantle is the inner layer of the mollusk that hangs like thin plates on both sides of the inner shell and covers the visceral mass, which contains almost all of the organs (Zainuddin et al., 2018). This genus has gills that serve as respiratory organs as well as collecting food such as

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algae, phytoplankton, and debris. These gills are covered in scales, which allow water to flow through the abdominal opening (Nugroho, 2022). Environmental pollution is defined as the introduction of living things, materials, energy, or other components into the environment, as well as changes in the environment's order, which cause the living environment to be damaged or unable to function. According to the United Nations.

Convention on the Law of the Sea - UNCLOS III, marine pollution is defined as a change in the marine environment, including estuaries (river estuaries), that causes negative consequences that can damage marine living resources, endanger human health, and disrupt activities at sea, including fishing and normal use of the sea, reduce the quality of seawater, and reduce the availability and benefits (Sihotang et al., 2018). Ocean and coastal water pollution can be classified into seven categories: industry, household waste, residential wastewater, shipping, agriculture, and aquaculture. Pollutants in the form of sediment, toxic metals, pesticides, nutrients, exotic organisms, pathogenic organisms, garbage, and oxygen-degrading substances that occasionally enter waste disposal reduce the amount of dissolved oxygen in seawater (Utami et al., 2018).

Oysters can be found around the waters of Lengkese Village, Takalar Regency and Coppo Village, Barru Regency. These oysters are a food ingredient for people in the region. The content of pathogenic bacteria in oysters in this region will have an impact on reducing the health status of the surrounding community. The impact that will be caused by the presence of pathogenic bacteria in oysters is causing digestive disorders, such as diarrhea and vomiting and other dangerous diseases. Based on this, the research carried out an analysis of the content of oyster pathogenic bacteria in Lengkese Village, Takalar Regency and Coppo Village, Barru Regency, South Sulawesi.

Method

Material

The following materials were used in this study: oyster meat samples, distilled water, Nutrien Agar (NA) medium, crystal violet solution, Lugol's solution, safranin, 95% alcohol, immersion oil, 70% alcohol, aluminum foil, umbrella paper, heat-resistant rubber, and wrapping paper.

Tool

The tools used were test tubes, Durham tubes, incubators, cups, petri dishes, erlenmeyer, beakers and measuring cups, volume pipettes, mortars and stampers, test tube racks, spirit lamps, autoclaves, object glass, cover glass, microscope, micro pipette, magnetic stirrer, stir bar, yellow tip, suction cup, hot plate and dropper pipette.

Sterilization of Tools

The tools will be washed with detergent, rinsed with distilled water, and finally dried. The tools then wrapped in parasol paper and sterilized in an autoclave for 15 minutes at 121°C pressure of 2 atm ((SNI, 2006).

Medium Manufacture and Sterilization

Nutrien Agar (NA) medium 2.3 g. NA medium was dissolved in 100 mL of distilled water, then heated to boiling and stirred until homogeneous. The medium was then sterilized in an autoclave for 15 minutes at 121°C and 2 atm pressure (SNI, 2006).

Biochemical Analysis

The Indole Test

One loop of culture was planted in the inward tryptone broth from the slanted NA culture. Incubated at 37°C for 24 hours. Following incubation, add 0.2-0.3 ml.

Each tube was filled with indole reagent, shaken, and left for a few minutes. A positive Indole reaction is indicated by the color of the surface forming a ring, while a negative Indole reaction is indicated by the color orange (SNI, 2006).

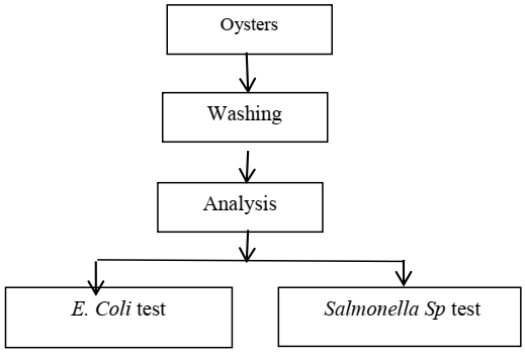

Figure 1. Flow diagram analysis of pathogenic bacteria in oysters

Discussion of the Findings

Biochemical Analysis

Basic principle is that enzymes peroxidase by microbes degrade carbohydrates and fats, and the metabolites can be seen visually in this case an additional indicator. Biochemical Test Results for Bacterial Content Salmonella sp. by Indole Testing on Oyster Meat Samples from Lengkese Village, Takalar Regency and Coppo Village Barru Regency seen in Table 1.

Table 1. Biochemical Test Results for Bacterial Content Salmonella sp oysters in Lengkese Village, Takalar Regency and Coppo Village Barru Regency

Location	Sample	Indole Test
Lengkese Village, Takalar Regency	Oyster meat	Negative
Coppo Village, Barru Regency	Oyster meat	Negative

Table 1 shows the results of bacterial testing. Salmonella species The Indole test results from the oyster samples from Coppo Village in Barru Regency and Lengkese Village in Takalar Regency were both negative. Bacterial Content Biochemical Test Results Table 2 shows the results of E. coliby's Indole testing on oyster meat samples from Lengkese Village, Takalar Regency and Coppo Village Barru Regency.

Table 2. Biochemical Test Results for Bacterial Conten E. colioysters, in Lengkese Village, Takalar Regency and Coppo Village, Barru Regency

Location	Sample	Indole Test
Lengkese Village, Takalar Regency	Oyster meat	Negative
Coppo Village, Barru Regency	Oyster meat	Positif

According to table 2, the oyster sample from Coppo Village, Barru Regency tested positive for bacteria E. colion. The oyster sample from Lengkese Village, Takalar Regency, tested negative for bacteria E. coli on the Indole test.

Result and Discussion

Salmonella sp. testing is shown in Table 1. The results from the oyster samples collected in Coppo Village, Barru Regency, and Lengkese Village, Takalar Regency, were both negative.

At the top of the sign Indole positive, a red alcohol layer will form as a ring (Effendi, 2020). This means that oyster samples from Ex. Coppo in Barru Regency were positive for E. coli bacteria, whereas samples from Lengkese Village in Takalar Regency were negative.

The solution contained a red ring, according to an Indole test on oysters in Coppo Village, Barru Regency. By using trypthopan enzymes, bacteria E. coli can produce indole from the breakdown of amino acids trypthopan. Erlichor Kovak's reagent can be used to detect indole production. Indole will react with the aldehyde in the reagent, turning it red. Coppo, Barru Regency, and oyster samples from Lengkese Village, Takalar Regency, were both negative on the Indole test.

Indole test on oysters in Coppo Village, red ring. The cherry red ring that is formed is due to the fact that E. coli bacteria can produce indole from the breakdown of tryptopan amino acids using tryptopan enzymes. Indole production will be detect district Barru showed that the solution contained a red using Erlich reagent or Kovak reagent. Indole will react with aldehyde in the reagent and give a red color.

A red alcohol layer will form like a ring at the top indicating positive Indole (Effendi, 2020). This means that the oyster samples from Ex. Coppo Barru Regency was positive for E. coli bacteria while samples from Lengkese Village, Takalar Regency were negative for E. coli bacteria.

E. coli bacteria in oysters in Coppo Village, Barru Regency is most likely due to the high concentration of organic matter (BOT) in the waters where oysters are found. Where it is known that bacteria can use organic materials for growth and development, so that the more organic materials used by bacteria, the more bacteria will grow. This is in accordance with the statement of Sidharta (2000) that material organic matter contains the elements needed by bacteria to grow, such as the elements contained in organic matter such as carbon, nitrate, phosphate, ammonia and various minerals that provide nutrients for microbial growth.

Oysters (Crassostrea cucullata) are animal biofilters that can absorb pathogenic bacteria in the water and extract both suspended and particulate organic matter (Rahayu et al., 2013). This animal is classified as a filter feeder, which is a type of animal that gets food by filtering air into its body (Putra et al., 2011). This way of eating causes various components of the ingredients to enter the body of the clam (Liliandari et al., 2013). Oysters can filter particles with a size of 0.1-50.0 mess from water bodies, then those with a particle size > 0.4 mess can filter up to 100%. Oysters are quite effective in improving water quality at a fairly high level of organic matter pollution and as a biofilter against (Safaringga et al., 2017). Mussels containing pathogenic bacteria are very harmful to human health when consumed immature (Astari et al., 2021).

Oysters are a type of mollusk that accumulates contaminants in their environment (Boudjema et al., 2022). Oysters have long been used as an indicator of water pollution in various geographic locations (Baumard et al., 1999; Beyer et al., 2017; Jin et al., 2008), because its ability to separate pathogenic chemicals and bacteria due to its stable population, reduced metabolic activity and immobility (Edge et al., 2014; Goldberg, 1975; Phillips, 1977).

Conclusion

The oyster meat sample of Tamaona Hamlet, Takalar Regency was negative for E. coli and Salmonella sp. While the oyster meat sample from Coppo Village, Barru Regency was positive for E. coli bacteria and negative for salmonella sp. Biochemical test on the Indole Test of oyster samples in the Coppo sub-district,

Barru Regency formed a cherry red ring which indicated that it contained E. coli positive. Oysters obtained from Lengkese Village, Takalar Regency had safety limits for E. coli and Salmonella sp bacteria contamination that met the standards and oysters from Coppo Village, Barru Regency had safety limits for E. coli bacteria contamination that did not meet the standards.

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Conflicts of Interest
No Conflicts of interest.

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