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Molecular Identification of Dominant Bacteria (16S rRNA) and Heavy Metal Contamination in Rono Dange, Central Sulawesi

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Abstract: Rono Dange is a traditional smoked anchovy product that has great economic value and potential as a local food. However, there are differences in quality, especially in terms of microbiology and food safety. This study aims to identify the dominant bacteria found in Rono Dange products and evaluate heavy metal contamination of lead (Pb), cadmium (Cd), and magnesium (Mg) as indicators of consumption safety. Rono Dange samples were obtained from five traditional processors in Lero Village and analyzed in the laboratory. Bacterial isolation and identification were performed using pure culture techniques, DNA extraction, 16S rRNA gene amplification using PCR, and DNA sequence analysis to determine the dominant species. Heavy metal content testing was performed using the AAS (Atomic Absorption Spectrophotometry) method in accordance with the SNI 7387:2009 standard. The results showed that the dominant bacteria found in the Rono Dange samples belonged to the genera *Bacillus, Staphylococcus, and Pseudomonas*, which play a role in the formation of the distinctive aroma but also indicate potential contamination if not properly controlled. The concentrations of Pb and Cd heavy metals in most samples were still below the SNI threshold, while Mg levels showed variations influenced by fuel and smoking duration. Overall, it was concluded that Rono Dange is still suitable for consumption, but it is necessary to standardize the smoking process and control the cleanliness of the production environment to ensure product quality and safety.

Keywords: 16S rRNA; Dominant Bacteria; Food Safety; Heavy Metals; Rono Dange.

Introduction

Indonesia is one of the largest fish producing countries in the world after China (Apriliana et al., 2024; Nurfadilah, et al., 2024) , with a production contribution of 24 million tons per year (Nawir et al., 2024). The output of the fisheries sector is an important part of a country's economic progress. The processing of fishery products must comply with certain criteria in order to produce high-quality and competitive fishery products in the global market (Bentley & Soebandrio, 2017; Pradianti et al., 2019).

Traditional smoked fish products are one form of fishery product processing that coastal communities in Indonesia have widely developed as a strategy to extend the shelf life of fish and increase their selling value. One of the distinctive and popular smoked anchovy products in Central Sulawesi is *Rono Dange,* a product of the Lero Village community in Donggala Regency. Rono dange is anchovies processed by wrapping them in leaves and smoking them with iron plates and coconut husks as a source of smoke and fuel. This product not only has economic value, but is also part of the local culinary cultural heritage that supports the food security of coastal communities. However, the smoking process,

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which is still carried out traditionally without adequate sanitation and quality control standards, has the potential to cause variations in product quality, both microbiologically and chemically. Several studies show that traditional smoked fish can contain pathogenic microbial contaminants and heavy metals that exceed safe limits if the processing is carried out with uncontrolled techniques and fuels (Fitriani et al., 2021).

The bioaccumulation of heavy metals occurs through the food chain, which can ultimately pose a risk to human health when consumed. As a result, fish are often used as an indicator of heavy metal pollution in aquatic ecosystems (Akan et al., 2012; Zaza et al., 2015).

Molecular characterization using the 16S rRNA gene is one effective method for identifying bacterial communities associated with smoked fish products. This method provides accurate taxonomic information about dominant microbes, both beneficial (such as lactic acid bacteria) and those that have the potential to cause contamination and quality deterioration (Schrader et al., 2024). Meanwhile, analysis of heavy metals such as lead (Pb), cadmium (Cd), and magnesium (Mg) is important to assess the safety level of product consumption, given that heavy metals are toxic and can accumulate in the human body (Ahmed et al., 2021).

Thus, a combination of molecular and analytical chemistry approaches is needed to provide a comprehensive picture of the quality and safety of *Rono Dange* products. The results of this study are expected to provide a scientific basis for the development of a quality assurance system for traditional smoked fish and to increase the competitiveness of local products from Central Sulawesi in the national market. This study aims to identify the dominant bacterial communities found in *Rono Dange* smoked fish products using 16S rRNA gene molecular analysis. Analyze the level of heavy metal contamination (Pb, Cd, and Mg) in *Rono Dange* products from several traditional processors in Lero Village, Donggalan Regency, and provide technical recommendations for improving traditional smoked fish processing standards based on the results of microbiological and heavy metal tests.

This study also has strong relevance in the context of science education and applied science learning, as the molecular identification and heavy metal analysis approaches employed can be utilized as laboratory-based learning materials, particularly in applied microbiology and food safety courses.

Method

Time and Place

This research was conducted from July to November 2025 at the BPOM Micro Laboratory in Palu and the Integrated Laboratory of Tadulako University in Palu.

Tools and Materials

The equipment used in this study included the following: writing instruments, label paper, markers, basins, stirrers, analytical scales, test tubes and racks, sterile Petri dishes, autoclaves, pH meter, drying oven, hotplate, Kjeldahl flask, Atomic Absorption Spectrophotometer (AAS), organoleptic assessment form, sterile microcentrifuge tubes, desiccator, PCR, thermocycler machine, sterile gloves, lab coats, masks. The materials used were rono dange fish, PCA (*Plate Count Agar)* media, MRSA / VRBA media, Potato Dextrose Agar (PDA) media, 70% alcohol, *DNA/RNA Shield,* concentrated nitric acid, perchloric acid, deionized water, and 70% ethanol.

Research Procedure

This stage was conducted through field observations and informal interviews with smoked fish producers to identify workflows, processing facility conditions, and sanitation practices that affect product quality (Sirait & Saputra, 2020). Along with the laboratory analysis process, critical contamination points in the traditional processing process were also identified to determine which parts of the process posed a high risk of product quality deterioration. After all data were collected, data analysis and interpretation were conducted, both descriptively and comparatively, against national food quality standards (Permata Nauli et al., 2024). The findings will be used to formulate recommendations for improving the traditional rono dange fish production process to be hygienic and meet standards, while still maintaining local wisdom. Sampling was then carried out at five smoked rono dange fish processors located in the research area of Lero Village, Donggala Regency, Central Sulawesi Province. Sampling was carried out in stages according to a schedule agreed upon with the rono dange processors, who represented each rono dange processing business unit.

Each processor was selected purposively, and then five packages of whole smoked rono dange, equivalent to 250 grams, were sampled from each processor. The samples were collected immediately after the production process was completed to maintain the freshness of the samples. The collected samples were then given an identification code according to the origin of the processor (e.g., RD-A, RD-B, RD-C, RD-D, and RD-E) and recorded on the sample collection observation sheet. To maintain quality, all samples were stored in sterile containers (labeled plastic bags) and placed in a cool box during transportation to the laboratory.

16S rRNA Molecular Characterization Testing (Purwandhani & Kurniawati. (2020)

Molecular characterization of dominant bacteria in smoked Rono Dange fish was conducted using the 16S rRNA gene as a molecular marker. This gene was selected due to the presence of conserved regions shared among all bacteria, as well as variable regions that enable species-level identification. Samples were collected from both the surface and inner flesh of the smoked fish, followed by bacterial DNA isolation using a silica column–based commercial extraction kit to obtain purified genomic DNA. (Valenzuela et al., 2021)

The extracted DNA was subsequently amplified by Polymerase Chain Reaction (PCR) using universal 16S rRNA primers (27F and 1492R). Amplification success was confirmed by 1% agarose gel electrophoresis, which revealed clear DNA bands of approximately ±1,500 bp. The purified PCR products were then subjected to DNA sequencing, and the resulting sequence data were analyzed using bioinformatics tools through BLAST-N comparison against the NCBI GenBank database to identify the dominant bacterial species present in the samples. (Gultom et al., 2025).

Heavy Metal Testing

Determining the levels of heavy metals Pb (Lead), Cd (Cadmium), and Mg (Magnesium) in food samples (smoked fish/fishery products) using the Atomic Absorption Spectrophotometry (AAS) method. The samples were processed through wet digestion using a mixture of strong acids to dissolve the organic matrix so that the metals were in ionic solution form. The sample solution is then analyzed with AAS at the specific wavelength of each metal. Heavy metals such as Lead (Pb), Cadmium (Cd), and Mercury (Hg) are toxic even in low levels. Consumption of contaminated smoked fish can pose chronic health risks, including kidney disorders, nerve damage, and cancer. The samples are processed through wet digestion using a mixture of strong acids to dissolve the organic matrix so that the metals are in ionic solution form. The sample solution is then analyzed with AAS at the specific wavelength of each metal (National Standardization Agency, 2009).

Sample Preparation

Weigh ± 2–5 g of a homogeneous smoked fish sample. Please place it in an acid-resistant Erlenmeyer flask.

Destruction Process

Add 10 mL of concentrated HNO_3. Heat on a hotplate at a temperature of ±120 °C until the brown smoke disappears. Add 2–5 mL of $HClO_4$ (if necessary), continue heating until the solution is clear. Cool, then dilute with distilled water to a specific volume (e.g., 50 mL) in a measuring flask. Filter if there is residue.

Measurement with AAS

Turn on the AAS, calibrate with standard solutions of Pb, Cd, and Mg at varying concentrations. Measure the samples at the following wavelengths: Pb: 217.0 nm, Cd: 228.8 nm, Mg: 285.2 nm. Record the absorbance of each sample.

Calculation

The metal concentration in the sample is calculated using the equation:

$$C_{sampel} = \frac{C_{bacaan} \times V_{akhir}}{W_{sampel}} \quad (1)$$

Explanation:
- C_sample = metal concentration in the sample (mg/kg)
- C_reading = concentration from AAS (mg/L)
- V_final = final volume of the solution (mL)
- W_sample = sample weight (g)

Result and Discussion

16S rRNA Molecular Characterization Testing

Molecular analysis using the **16S rRNA** gene marker was able to identify the dominant bacteria in each Rono Dange processor. The results showed variations in microflora. The data from the 16S rRNA Molecular Characterization Test of 5 Rono Dange processors can be seen in Table 1.

Table 1. Comparison of 5 Rono Dange Processors Based on 16S rRNA

Rono Dange Processor	Molecular Analysis Results (16S rRNA)	Identification of Dominant Bacteria
Processor 1	The 16S rRNA gene fragment was successfully amplified (±1500 bp) with homology >98%	*Bacillus subtilis*
Processor 2	Clear amplification of 16S rRNA, homology >97%	*Staphylococcus aureus*
Processor 3	The 16S rRNA sequence shows 99% homology	*Pseudomonas aeruginosa*
Processor 4	Analysis results show a complete 16S rRNA fragment, 98% homology	*Lactobacillus plantarum*
Processor 5	The gene fragment was successfully amplified, with 97–99% homology	*Escherichia coli*

Molecular characterization results using the 16S rRNA gene marker indicate differences in the microbial flora composition of Rono Dange from five different processors. In Processor 1, the identified bacteria were *Bacillus subtilis*, which are generally proteolytic and can support the formation of a distinctive flavor in smoked fish products, making them relatively harmless. Meanwhile, in Processor 4, the dominant bacteria were *Lactobacillus plantarum*, which are lactic acid bacteria that have the potential to provide a protective effect because they can inhibit the growth of pathogenic bacteria.

However, *Staphylococcus aureus* was detected in Processor 2, indicating possible contamination from the processing environment or human contact, thus requiring special attention in terms of sanitation and hygiene. The results from Processor 3 showed the presence of *Pseudomonas aeruginosa*, a bacterium known as a spoilage bacterium, whose presence can accelerate product deterioration. Furthermore, Processor 5 showed the presence *of Escherichia coli,* which is an indicator of fecal contamination, possibly originating from unclean water or equipment. This is a critical finding because *Escherichia coli* can be harmful to consumer health.

In general, these differences in microflora profiles reflect variations in processing and sanitation practices among Rono Dange processors. Products from processors with positive bacteria (*Bacillus* and *Lactobacillus*) are of better quality and safer, while other processors have pathogenic bacteria that can reduce quality and pose a health hazard. Therefore, simple measures such as improving environmental hygiene, using clean water, hygienic equipment, and controlling temperature during the production process are needed to reduce the risk of contamination and ensure product safety in accordance with food standards (Nurfadilah et al., 2024).

Several studies on smoked fishery products have shown the diversity of microflora, both beneficial and pathogenic. For example, *Bacillus subtilis* is often found in fermented and smoked products, playing a role in producing proteolytic enzymes that can enhance the taste and safety of products (Elbarbary et al., 2023). Similarly, *Lactobacillus plantarum* is a lactic acid bacterium (LAB) known to produce antimicrobial compounds such as bacteriocins, which inhibit the growth of pathogenic bacteria (*Escherichia coli, Salmonella, Staphylococcus aureus)* in food products (Mailoa et al., 2019). Thus, the results of the study on Rono Dange show bacterial variations in each processor, consistent with previous literature findings. The presence of beneficial bacteria such as *Bacillus* and *Lactobacillus* can support product quality. In contrast, the presence of pathogenic bacteria emphasizes the importance of implementing Good Manufacturing Practices (GMP) and Sanitation Standard Operating Procedures (SSOP) in the processing of rono dange fish (National Standardization Agency, 2013).

On the other hand, the presence of pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli* in smoked fish products has been reported in various studies as an indicator of poor hygiene during processing (Maillet et al., 2021). *Staphylococcus aureus* can produce enterotoxins that are harmful to consumer health (Gultom et al., 2025), while *Escherichia coli* is often used as an indicator of fecal contamination and poor sanitation (Azizah & Widodo, 2023). *Pseudomonas aeruginosa* is also commonly found in fish products as a spoilage bacterium that can accelerate sensory quality deterioration, particularly aroma and texture (Zees et al., 2024). Overall, these results emphasize the importance of standardizing processing and sanitation processes to maintain the quality and safety of Rono Dange.

The presence of potential pathogenic bacteria, such as *Staphylococcus aureus* and *Escherichia coli*, indicates molecular-level detection of bacterial DNA and does not directly reflect an immediate health risk to consumers. Health risk assessment should consider microbial load, virulence characteristics, and compliance with maximum allowable limits established by food safety standards. Therefore, these findings are more appropriately interpreted as indicators of processing hygiene rather than definitive evidence of product unsafety.

Heavy metal testing

The results of heavy metal testing from 5 Rono Dange processors can be seen in Table 2.

Table 2. Heavy Metal Test Results on Rono Dange

Processor	Test Results Pb (mg/kg)	Cd Test Results (mg/kg)	Mg Test Results (mg/kg)	SNI 7387:2009 Standard*
Processor 1	0.04	0.01	52.3	Pb ≤ 0.3; Cd ≤ 0.1
Processor 2	0.06	0.02	49.8	Pb ≤ 0.3; Cd ≤ 0.1
Processor 3	0.10	0.03	55.1	Pb ≤ 0.3; Cd ≤ 0.1
Processor 4	0.07	0.01	51.0	Pb ≤ 0.3; Cd ≤ 0.1
Processor 5	0.12	0.05	53.7	Pb ≤ 0.3; Cd ≤ 0.1

Source: Primary Data, 2025

Processor 1 The test results show that the Pb (0.04 mg/kg) and Cd (0.01 mg/kg) contents are very low and far below the SNI 7387:2009 threshold. The Mg content (52.3 mg/kg) is within the natural range for smoked fish. This indicates that the products from Processor 1 are relatively safe and of good quality, with indications that the production process is sufficiently clean and controlled. Processor 2 The Pb (0.06 mg/kg) and Cd (0.02 mg/kg) values are still low and safe for consumption. The Mg content (49.8 mg/kg) is slightly lower than that of Processor 1, but still within the normal range. This difference may be due to variations in raw materials or smoking intensity. In general, the results show that food safety is maintained. Processor 3 The Pb (0.10 mg/kg) and Cd (0.03 mg/kg) values are higher than the previous processors, although still below the maximum limit for heavy metal contamination according to SNI.

The Mg content (55.1 mg/kg) is relatively high, which is good from a nutritional point of view. However, this higher heavy metal content needs to be taken into consideration, as it may originate from the fuel used for smoking or environmental factors during processing. Processor 4's Pb (0.07 mg/kg) and Cd (0.01 mg/kg) levels remain safe. The Mg content (51.0 mg/kg) is balanced, supporting the nutritional profile of smoked fish. Products from Processor 4 show fairly good quality, with low heavy metal contamination. This indicates relatively more hygienic processing practices. Processor 5 Pb (0.12 mg/kg) and Cd (0.05 mg/kg) were the highest among all processors, although still below the SNI threshold. This indicates a potential risk if processing practices are not improved. The Mg content (53.7 mg/kg) remains within the natural range. Products from Processor 5 require more attention to hygiene, fuel selection, and equipment sanitation in order to reduce heavy metal content (Fitriani et al., 2021).

Food safety in smoked fish products is significantly influenced by the potential for heavy metal contamination during the production process. According to SNI 7387:2009 concerning Maximum Limits of Heavy Metal Contamination in Food, the maximum permissible Pb level in fish products and their processed products is 0.3 mg/kg. In comparison, Cd is limited to 0.1 mg/kg (Ahmed et al., 2021). Test results on Rono Dange showed that all samples from five processors were still below these thresholds, meaning the products can be categorized as safe for consumption.

Pb and Cd contamination in smoked fish generally originates from the aquatic environment, residues from smoking fuel, and the equipment used (Arub Arisma et al., 2023). The traditional smoking process using wood or shells without strict control has the potential to increase the risk of contamination, mainly if the fuel contains heavy metals (Aristawati et al., 2024). Therefore, the variation in Pb and Cd levels in Rono Dange's test results may reflect differences in processing practices and environmental conditions of each processor.

Meanwhile, magnesium (Mg) is an essential mineral that is naturally found in fish. The Mg content in smoked fish is relatively stable and contributes to the nutritional value of the product. According to Elbarbary et al. (2023), magnesium plays an important role in enzymatic function and metabolism. Variations in Mg content between processors are more influenced by the type of fish used and the level of processing, rather than contamination factors. In general, previous studies emphasize the importance of monitoring heavy metals in traditional smoked fishery products. (Ogunyebi et al., 2025) report that smoked fish produced in Nigeria shows variations in Pb and Cd levels between processing locations, with some approaching the threshold limit. Similar conditions were found by Fitriani et al. (2021) in smoked fish in Indonesia, where environmental factors and traditional smoking methods were the main determinants of contamination levels.

Thus, the results of this study are consistent with the existing literature, namely that although Rono Dange products are still safe for consumption, simple controls over fuel sources, raw material quality, and equipment hygiene are very important to maintain consistency in quality and food safety.

Conclusion

The results of this study indicate that traditional smoked fish products from Rono Dange in Lero Village, Donggala Regency, have varying microbiological characteristics and heavy metal content among processors. Molecular analysis of the 16S rRNA gene successfully identified the presence of several dominant bacteria, including Bacillus subtilis, Pseudomonas aeruginosa, and Lactobacillus plantarum. Heavy metal content analysis showed that lead (Pb) levels between 0.04 and 0.12 mg/kg and cadmium (Cd) levels between 0.01 and 0.05 mg/kg in some samples were still within safe limits according to SNI 7387:2009. Several samples were close to the specified threshold. Magnesium (Mg) content was relatively high at 49.8–55.1 mg/kg, but still considered safe for consumption.

The contribution of this study to the development of applied science and food safety education can serve as a case study for laboratory-based learning, particularly in courses on applied microbiology, food safety, and fisheries product processing.

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Author Contributions
S.S.M: Developing ideas, overseeing data collection, analyzing, writing, reviewing, responding to reviewers' comments; N.F.: analyzing data, overseeing data collection, reviewing scripts, and writing.

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Conflicts of Interest
The authors declare no conflict of interest.

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