

Uncovering the Endogenous Bacteria Involved in the Synthesis of Formaldehyde in Tiger Grouper Fish (*Epinephelus fuscoguttatus*)

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Abstract. This study aimed to isolate and identify specific bacteria responsible for the conversion of trimethylamine oxide (TMAO) into formaldehyde in tiger grouper (*Epinephelus fuscoguttatus*). The gastrointestinal tract of tiger grouper specimens was sampled, and bacterial colonies were isolated using nutrient agar (NA) and screened on TMAO media. Bacterial isolates capable of producing formaldehyde were identified. The implications of these findings extend to food safety, scientific research, medical diagnostics, and industrial applications. Further investigation is needed to elucidate the enzymatic pathways and genetic factors associated with formaldehyde production in these bacteria. Evaluating the antibacterial compound production capability revealed inhibitory zones against *Escherichia coli*, indicating the potential for bactericidal properties. Gram staining and scanning electron microscopy (SEM) were employed to characterize the formaldehyde-producing bacteria, revealing their gram-positive nature and rod-shaped morphology. This study provides insights into the role of specific bacteria in formaldehyde production, paving the way for future research and applications in various fields.

Keywords: Formaldehyde, Tiger grouper, *Epinephelus fuscoguttatus*

INTRODUCTION

Formaldehyde, known as the simplest and most highly reactive aldehyde group, exists as a gas called formaldehyde and as a liquid known as formalin [1]. Inhaling formaldehyde can lead to respiratory tract irritation, while contact with the skin can cause burns, allergic reactions, and, if ingested, it can even induce cancer in humans. Elevated levels of formaldehyde in the body can react chemically with cellular substances, resulting in suppressed cell function, cell death, and damage to organs [2]. In recent years, the misuse of formaldehyde compounds, particularly formalin solution, as a food preservative has become increasingly common. Fishery products often employ formaldehyde to extend the shelf life and maintain the freshness of fish [3]. Fish is more prone to rapid spoilage and decay than other animal protein sources. The deterioration of fish begins with autolysis, followed by microbiological, physical, and chemical degradation. Among these, microbiological spoilage is the most significant in both fresh and processed fish [4], [5]. Microbiological spoilage intensifies after the completion of biochemical deterioration (rigor mortis). Bacteria that initially reside in the fish's gills, stomach, and skin infiltrate the muscles, breaking down energy sources such as proteins, fats, and carbohydrates into harmful compounds [6].

It is important to note that not all instances of formaldehyde in fish are the result of intentional addition. Instead, volatile compounds can form during the decay process (deterioration) of protein- and fat-rich foods like fishery products [7], [8]. For instance, formaldehyde can be produced in fish tissue as it undergoes deterioration. According to a recent study, tiger grouper (*Epinephelus fuscoguttatus*) was found to generate formaldehyde as a trimethylamine derivative after 20 hours of storage at room temperature, with concentrations ranging from 2.4 to 3.0 ppm [9]. Furthermore, another study reported that grouper fish produces formaldehyde during decay when stored at cold temperatures (0-4°C) for ten days [10].

The formation of formaldehyde in fish can be attributed to two types of enzymes: endogenous enzymes produced by the fish's body itself and exogenous enzymes produced by bacteria. Exogenous enzymes, specifically trimethylamine oxide reductase (TMOase), play a role in

reducing trimethylamine oxide (TMAO) to trimethylamine (TMA), dimethylamine (DMA), and formaldehyde (FA) [7]. Bacteria play a significant role in the production of formaldehyde during the decomposition (deterioration) process of fish. Therefore, this study aims to identify the specific bacteria involved in the conversion of TMAO into formaldehyde in tiger grouper (*Epinephelus fuscoguttatus*).

Formaldehyde is a chemical that should be avoided in food, including fish. However, it appears that naturally occurring groupers (*Epinephelus fuscoguttatus*) produce formaldehyde during the decay process [8]. The enzyme responsible for this production is TMAOase, which can be generated by various bacteria present in groupers. This study aims to determine the bacteria involved in the production of formaldehyde during the deterioration process of tiger grouper [9].

The purpose of this study is to isolate and identify the specific bacteria that play a crucial role in the conversion of TMAO into formaldehyde in tiger grouper (*Epinephelus fuscoguttatus*). By conducting this research, valuable information regarding the types of bacteria involved in the formation of formaldehyde in tiger grouper can be obtained.

The findings of this study are expected to contribute to a better understanding of the microbial processes that occur during fish deterioration and provide insights into the mechanisms of formaldehyde production in fish. This knowledge can be utilized in various ways, such as developing effective preservation techniques and preventing the inadvertent presence of formaldehyde in fishery products.

Moreover, identifying the bacteria responsible for formaldehyde formation can have significant implications for food safety and public health. By targeting these specific bacteria, appropriate measures can be implemented to control and minimize the risk of formaldehyde contamination in fish, thereby ensuring the safety and quality of seafood products.

METHODOLOGY

2.1 Research Location and Duration

This research will be conducted at the Research Laboratory of the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Syiah Kuala University in Banda Aceh. The research will span from December 2012 to September 2013.

2.2 Instruments and Materials

2.2.1 Instruments

The following instruments will be used: petri dishes, micro pipettes, autoclaves, incubators, ose wires, mortars and pestles, microscopes, spectrophotometers, centrifuges, a set of Scanning Electron Microscope (SEM) equipment, a set of electrophoretic devices, and a set of Polymerase Chain Reaction (PCR) tools.

2.2.2 Materials

The materials utilized in this research include tiger grouper (*Epinephelus fuscoguttatus*) obtained from Ulee Lheu grouper germplasm in Banda Aceh, Trimethylamine Oxide (TMAO), peptone, Beef Extract, NaCl, Yeast Extract, agar, Nutrient Agar (NA) media, sea water, *E. coli* bacteria, formaldehyde, Chloroform, Isoamyl alcohol, isopropanol, SDS, and EDTA.

2.3. Research Procedure

2.3.1 Sample Preparation

Samples of tiger grouper (*Epinephelus fuscoguttatus*) will be collected from Krapu fish sprouts in Ulee Lheu. Live and fresh fish will be transported to Banda Aceh. The fish will be euthanized and left to stand for 18-20 hours. The outer surface of the fish will be disinfected to eliminate microbes. Aseptic surgery will be performed to obtain a portion of the stomach contents, which will be washed with sterile water and crushed using a sterile mortar and pestle.

2.3.2 Bacterial Isolation

Bacterial isolation will be conducted using Nutrient Agar (NA) media. One gram of homogenized spleen and fish kidney will be suspended in 10 mL of sterile sea water. Dilution series will be prepared, and each dilution will be plated on NA media and incubated at 35°C for 24-48 hours. Single colonies will be selected from the NA media and transferred to TMAO media using sterile toothpicks. Each bacterial culture obtained will be suspended in sterile aquades to obtain a bacterial isolate suspension.

2.3.3 Testing the Ability to Produce Antibacterial Compounds

The selection of bacterial colonies with antibacterial activity will be carried out using diffusion techniques. *E. coli* bacteria suspended in TMAO media will be poured into sterile petri dishes and allowed to solidify. Holes will be made in the TMAO media using a sterile instrument. Bacteria from the isolated cultures suspended in sterile water will be inserted into the holes. The petri dishes will be incubated at 35°C and observed periodically. The presence of inhibitory zones will indicate antibacterial activity. *E. coli* bacteria will serve as the bioindicator and negative control, while 37% formaldehyde will be used as the positive control. Qualitative identification of formaldehyde will be conducted using the Schiff reagent.

2.3.4 Identification of Antibiotic-Producing Bacteria (Formaldehyde)

- Gram Staining

Gram staining analysis was conducted at Syiah Kuala University's Faculty of Veterinary Medicine. Bacterial samples were prepared as thin suspensions on slides and dried, followed by fixation using a Bunsen burner. Violet crystal dye was applied as drops and allowed to stand for 30 seconds before being washed with running water. The slide was then treated with Lugol's solution (composed of Iodine, Potassium Iodine, and distilled water in a ratio of 1:2:300) for 30 seconds and washed again. Next, the preparation was rinsed with 96% alcohol for 2 seconds to remove the dye, followed by rinsing with distilled water. One drop of counterstain (safranin) was added to the preparation and left for 1 minute. The slide was rinsed with distilled water until the color disappeared and then dried for observation under a microscope [10], [11].

- Identification with SEM

The identification of bacterial morphology using Scanning Electron Microscope (SEM) was performed at Syiah Kuala University's Faculty of Engineering. The SEM sample preparation involved the following steps: Bacterial cells were separated from their supernatants through centrifugation at 6000 g. The resulting cell pellets were washed three times with phosphate saline buffer (150 mM phosphate buffer, pH 7.2, with 0.85% NaCl). Subsequently, 1 mL of 0.25% glutaraldehyde in 0.15 M sodium phosphate buffer (pH 7.2) was added to the cell pellets and incubated at room temperature for 30 minutes. The cell pellets were then incubated overnight at 4°C, followed by three washes with 0.15 M sodium phosphate buffer (pH 7.2). The mixture of cell pellets was centrifuged at 6000 g, and the pellets were washed with consecutive ethanol concentrations of 30%, 50%, 70%, 80%, and 90% for 10 minutes each [12], [13]. After centrifugation at 6000 g, the washing buffer was discarded, and the cell pellets were washed with 90% ethanol. Finally, the cell pellets were washed with 100% ethanol and incubated for 1 hour. The varying ethanol concentrations during washing were employed to remove residual water from the cells. The samples were observed under a Scanning Electron Microscope (SEM).

- Isolation of Chromosomal DNA

The isolation of chromosomal DNA was carried out using an enzymatic-based lysis method. Cell pellets were suspended in 200 µL of Tris buffer (pH 8.0) containing 8 mg/mL lysozyme and incubated at 37°C for 1 hour. Subsequently, the cells were lysed by adding 200 µL of lysis buffer containing 2% SDS and 200 mM EDTA (pH 8.0). The lysis process was conducted at 50°C for 30 minutes. Then, 150 µL of cold C solution (comprising 60 mL 5M CH₃COOH, 11.5 mL glacial acetic acid, and 28.65 mL H₂O) was added, followed by vortexing for 10 seconds. The mixture was incubated on ice for 5 minutes and then centrifuged at 8000 x g for 20 minutes. The supernatant was transferred to a new microcentrifuge tube, and 300 µL of chloroform isoamyl alcohol (24:1) was added. After vortexing, the mixture was centrifuged at 8000 x g for 60 minutes. The upper layer was transferred to a new tube, and the addition of chloroform:isoamyl alcohol (24:1) was repeated twice. The DNA was then precipitated by adding 0.6 mL of isopropanol and incubating at room temperature for 60 minutes. After centrifugation at a speed of 8000 x g for 15 minutes, the DNA pellets were washed three times with 70% ethanol, dried, and finally dissolved in ultrapure water [11], [14].

- DNA amplification using PCR (Polymerase Chain Reaction)

To perform DNA amplification using the Polymerase Chain Reaction (PCR), a preparation stage was necessary [14], [15].

Table 3.1. PCR (Polymerase Chain Reaction) sample reaction composition

Material	Volume (µL)
H ₂ O (pure)	20 µL
Primer com 1 F	1 µL
Primer com 1R	1 µL
DNA chromosome	3 µL
Amplitaq	25 µL

PCR conditions are set as follows:

Phase 1		Phase 2 repeated 30 times			Phase 3 Cooling
94 °C	94 °C	50 °C	72 °C	72 °C	4 °C
5 minutes	1 minute	1 minute	2 minutes	10 minutes	15 minutes

▪ DNA electrophoresis

The initial step involves the preparation of a 50x TAE buffer. This is achieved by combining 10 mL of EDTA pH 8.0, 5.71 mL of glacial acetic acid, and 24.2 g of tris bases. Subsequently, an agarose gel is prepared by weighing 0.6 g of agarose and mixing it with 60 mL of 1x TAE buffer. The mixture is then heated until boiling, and 2 µL of red gel dye is added. Once the gel solution is obtained, it is poured into a mold and allowed to solidify. Gel wells are then created for sample loading[14], [16], [17].

Each well is filled with the following components:

- 1 kb DNA ladder: A combination of 1 µL of DNA ladder, 1 µL of loading buffer, and 4 µL of water.
- PCR results: A mixture of 5 µL of PCR results and 1 µL of loading buffer.
- Negative control: A combination of 5 µL of water and 1 µL of loading buffer.

Electrophoresis is conducted under specific conditions, including a voltage of 60 volts, a current of 2 A, and a duration of 1.15 hours.

▪ DNA Sequencing

The DNA sequencing procedure was performed at the Microgen laboratory located in South Korea.

RESULTS AND DISCUSSION

3.1. Identification of Formaldehyde-Producing Bacteria

In this study, we focused on the identification of bacteria that are responsible for the production of formaldehyde. The target bacteria were isolated from the gastrointestinal tract of tiger grouper (*Epinephelus fuscogottatus*) specimens that were stored at room temperature for a specific duration of 20 hours. This time interval was selected based on previous research, which revealed that formaldehyde naturally accumulates in tiger grouper within the range of 18-20 hours[8].

To initiate the isolation process, the homogenized contents of the fish's stomach were suspended in sterile distilled water and subsequently subjected to serial dilutions up to a dilution factor of 103. This dilution step was performed to facilitate the isolation of individual bacterial colonies, enabling easier handling and characterization. Nutrient Agar (NA) was employed as the growth medium during the initial cultivation of bacteria. NA medium is a commonly used non-selective medium that provides the necessary nutrients for bacterial growth, including carbon sources for cellular structures and energy, nitrogen sources (proteins) for protoplasm and cell wall formation, as well as essential mineral salts to support overall growth[5], [6].

The isolated bacterial colonies were then subjected to further screening by transferring them onto Trimethylamine Oxide (TMAO) media. The objective of this screening process was to identify bacterial isolates capable of producing formaldehyde (Figure 1.1). Formaldehyde synthesis occurs through the enzymatic breakdown of trimethylamine oxide (TMAO) into trimethylamine (TMA), formaldehyde (FA), and dimethylamine (DMA) in equimolar proportions. This enzymatic reduction is facilitated by TMAO reductase during the spoilage of fish. The reaction can be represented as follows[18][19][20]:



The identification of formaldehyde-producing bacteria holds significant implications in various fields. In the food industry, the presence of formaldehyde can impact food safety and quality[4], [21]. Understanding the bacteria responsible for its production can aid in the development of

strategies to control and prevent formaldehyde accumulation in fish products[3], [6], [22]. Additionally, formaldehyde has diverse applications in scientific research, medical diagnostics, and industrial processes. Identifying and studying the bacteria involved in its production can contribute to advancements in these areas[5], [12], [22]. Furthermore, the findings from this study can serve as a foundation for future investigations exploring the enzymatic pathways and genetic mechanisms underlying formaldehyde production in bacteria[11], [23].

The research successfully isolated formaldehyde-producing bacteria from the gastrointestinal tract of tiger grouper. The initial isolation process involved the suspension and dilution of fish stomach contents, followed by the cultivation of individual bacterial colonies on Nutrient Agar (NA) medium. Subsequent screening on Trimethylamine Oxide (TMAO) media allowed for the identification of isolates capable of producing formaldehyde[6], [19], [24]. The implications of these findings extend to food safety, scientific research, medical diagnostics, and industrial applications. Further investigations are warranted to elucidate the enzymatic pathways and genetic factors associated with formaldehyde production in these bacteria[2], [25].

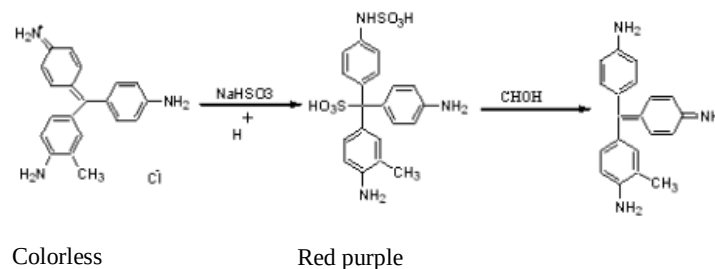
3.2. Evaluation of Antibacterial Compound Production Capability

The assessment of the capability to produce antibacterial compounds was conducted employing the agar well diffusion method. TMAO media were prepared through the pour method, wherein *E. coli* bacteria were incorporated into the TMAO media prior to solidification. This technique ensured that the bioindicator bacteria (*E. coli*) could permeate the entirety of the media, rather than being confined to the surface. The agar well diffusion method was selected to enable the diffusion of the isolated bacteria into the media, reaching the bottom and inhibiting the growth of *E. coli* bacteria[10][12][16].

Out of the 200 bacterial colonies exhibiting growth on the TMAO media, 50 isolates were chosen for further examination to determine their capacity to produce formaldehyde. However, not all colonies displayed positive outcomes in the formaldehyde production test. Positive results were indicated by the presence of inhibitory zones, where the bacterial isolates impeded the growth of *E. coli* bacteria by acquiring additional nutrients for formaldehyde production [2], [26]. A 37% formaldehyde solution was employed as a positive control due to its effective bactericidal properties against *E. coli*, resulting in the formation of an inhibition zone[10], [27]. *E. coli* bacteria were used as negative controls to verify their lack of ability to produce formaldehyde. The growth results of the bacterial isolates that formed inhibitory zones are presented in Figure 1.2.

It was observed that not all bacterial isolates subjected to testing exhibited the capability to produce formaldehyde[28]–[30]. Approximately 30% of the 300 isolates failed to produce formaldehyde, as evidenced by the absence of growth of the isolates that dominated the *E. coli* bacteria (Figure 1.3).

To confirm the formaldehyde production ability of the bacterial isolates, a qualitative formaldehyde test was conducted using Schiff reagent. Schiff reagent, composed of Fuchsin dye, Sodium Hydrogen Sulfite, and Hydrogen chloride 2 M, undergoes a color change in the presence of aldehydes. A slight presence of aldehydes induces a purplish-red color change (Figure 1.4). The reaction occurring in the qualitative test for aldehydes with Schiff reagent is as follows[31]:



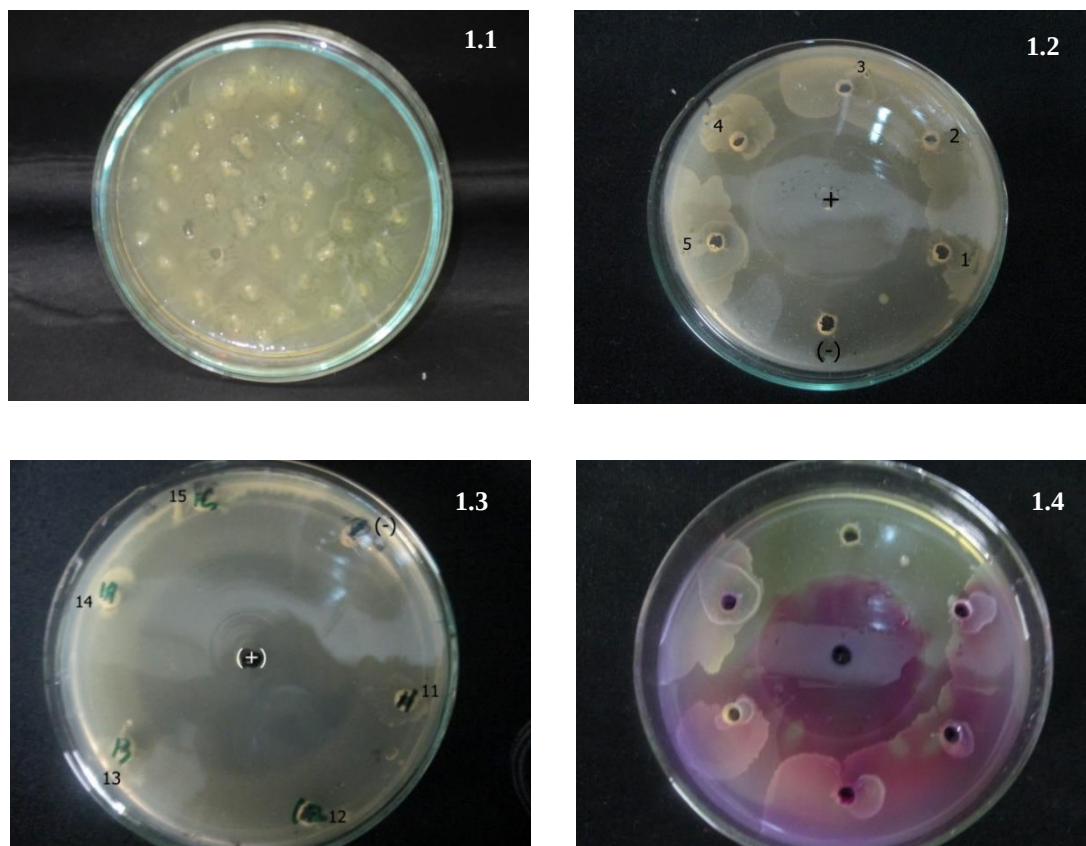


Figure 1. Growth of bacterial colonies on TMAO media: The initial stage of bacterial growth in TMAO media (1.1); The findings regarding the development of isolated bacteria that create zones of inhibition (1.2); The absence of growth in the isolated bacteria that outcompete *E. coli* bacteria (1.3); and The reactions that take place in qualitative tests of aldehydes with Schiff reagents (1.4).

3.3. Identification of Formaldehyde-Producing Bacteria

The identification and characterization of formaldehyde-producing bacteria are crucial for understanding their role in various biological processes and environmental systems. In this study, multiple methods were employed to elucidate the characteristics of these bacteria, including gram staining, Scanning Electron Microscope (SEM) analysis, and 16S rRNA identification.

3.3.1. Gram Staining

Gram staining is a widely used technique in microbiology that allows the differentiation of bacteria into gram-positive and gram-negative groups based on their cell wall composition. In this study, gram staining was performed to determine the gram nature and morphological features of the isolated bacteria capable of producing formaldehyde. The results, as depicted in Figure 2, demonstrated that these bacteria exhibited a gram-positive phenotype and had a rod-shaped morphology (bacilli). The retention of purple crystalline dyes by the bacterial cells indicated their ability to maintain the crystal violet stain, resulting in a distinctive purple color when observed under a microscope [6], [11].

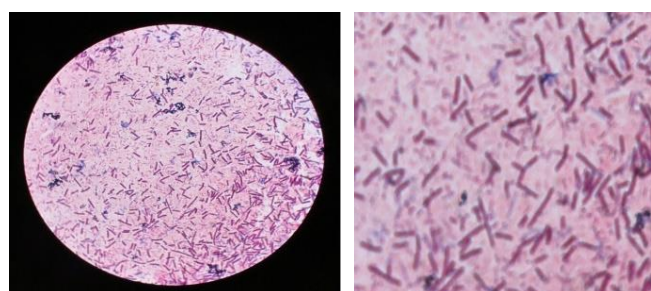


Figure 2. Gram staining reveals the presence of formaldehyde-producing bacteria that exhibit characteristics of being gram-positive and rod-shaped (bacilli)

3.3.2. Scanning Electron Microscope (SEM)

SEM analysis provides high-resolution imaging of the surface morphology and structure of microorganisms. In this study, SEM was employed to obtain a more detailed visualization of the isolated bacteria's morphology. Prior to SEM analysis, careful preparation of the bacterial cells was conducted to ensure cell death while preserving their structural integrity. However, due to the absence of gold (Au) coating on the bacterial cells, the SEM results exhibited limited resolution and reflected light. Nevertheless, at a magnification of 10,000x (Figure 3), it was still possible to identify the rod-shaped morphology of the isolated bacteria. Further characterization and classification of the bacterial genus were subsequently performed using 16S rRNA analysis [4], [32], [33]. The discussion of the 16S rRNA identification and its implications for the identification of formaldehyde-producing bacteria will be presented in the subsequent sections.

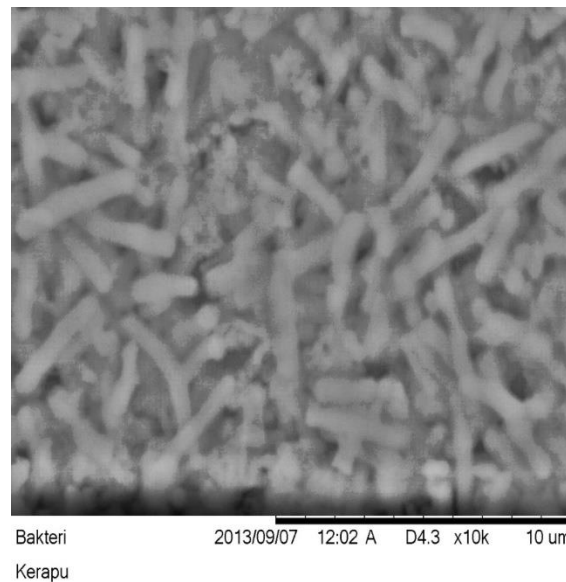


Figure 3. The morphology of the isolated bacteria was examined using SEM at a magnification of 10,000x

3.3.3. Analysis of 16S rRNA

The analysis of 16S rRNA was conducted in several stages, including DNA isolation, Polymerase Chain Reaction (PCR), DNA electrophoresis, and DNA sequencing. DNA isolation was performed using the lysis method, aiming to separate DNA from other cellular components such as proteins, fats, and carbohydrates. The lysis process involves breaking the cell wall using lysis buffers, which typically consist of a mixture of SDS and EDTA. The addition of EDTA and SDS facilitates cell membrane lysis and reduces the activity of nucleases, which are enzymes that degrade DNA. During the lysis process, RNA and proteins are released through the cell membrane, while DNA remains within the cell [16].

Polymerase Chain Reaction (PCR) is employed to amplify the isolated bacterial DNA. The primers used in this study were COM primers (F: CAGCAGCCGCGGTAATAC; R: CCGTCAATTCCTTTGAGTTT [15], [17], [25].

PCR involves several steps, starting with DNA denaturation at 94°C for 5 minutes in the first cycle and 1 minute in subsequent cycles. Denaturation involves heating the double-stranded DNA to separate it into single strands by breaking the hydrogen bonds between the chains. Subsequently, the temperature is lowered to facilitate primer binding to the single-stranded DNA, a step known as annealing. Annealing occurs at 50°C for 1 minute. The next step is elongation, during which the DNA polymerase enzyme extends the DNA chain and synthesizes new copies of DNA. The elongation temperature is set at 72°C, which is optimal for the DNA polymerase enzyme, with a time of 2 minutes for the initial cycle and 10 minutes for the final cycle. The

process concludes with cooling at 4°C for 15 minutes. After PCR, DNA amplification occurs, and the amount of amplified DNA can be visualized through DNA electrophoresis [25], [26], [34].

DNA electrophoresis was performed at a voltage of 60 V for 90 minutes, utilizing agarose gel as the medium. The resulting DNA bands appear as distinct bands (Figure 4), indicating successful DNA isolation. To determine the identity of the isolated bacterial DNA, DNA sequencing is carried out.

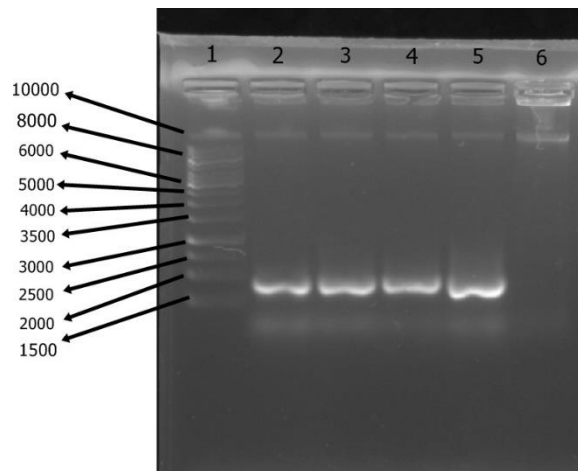


Figure 4. DNA electrophoresis was performed at 60 V for 90 minutes using agarose gel, resulting in well-separated DNA bands.

The DNA sequencing analysis revealed peaks that were less distinct and exhibited overlapping patterns (Figure 5). This phenomenon could be attributed to the limited purity of the isolated bacteria, resulting in overlapping peaks in the sequencing data. Dilution of the less abundant bacterial isolates may have contributed to their impurity, potentially hindering the attainment of single-cell identification [35], [36]. To assess the presence or absence of bacteria similar to the isolated strains, a DNA blast analysis was conducted using the NCBI website. The results of the DNA blast indicated a maximum similarity of 78% between the isolated bacteria and other known bacteria. Bacteria exhibiting 78% or higher similarity to the isolated strains were identified as uncultured strains. The comprehensive DNA blast results are presented in Figure 6.

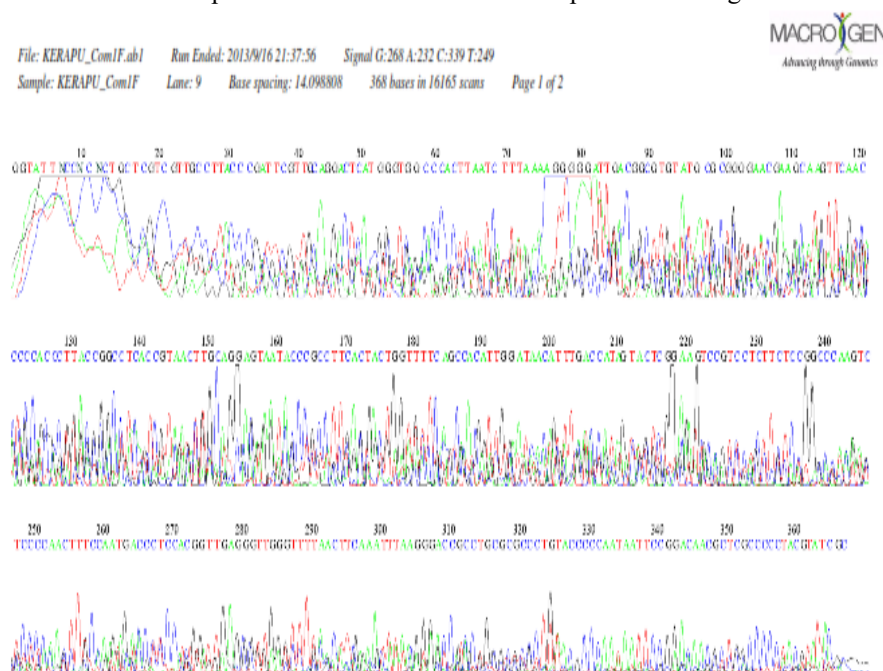


Figure 5. The DNA sequencing data exhibited peaks

To investigate the evolutionary relationship between the isolated bacteria and other bacteria sharing similarities, a phylogenetic tree was constructed. The phylogenetic tree was generated using the Mega 5 application, and the results are displayed in Figure 6. Surprisingly, the phylogenetic analysis revealed that the isolated bacteria, referred to as grouper bacteria, did not demonstrate an evolutionary relationship with the bacteria displaying similarities. This outcome suggests that the isolated DNA may originate from previously uncharacterized or uncultured bacteria. In order to verify this, the bacterial isolates were subjected to further purification procedures to obtain single bacterial cells, followed by a more precise analysis of the 16S rRNA gene to acquire more accurate and reliable results.

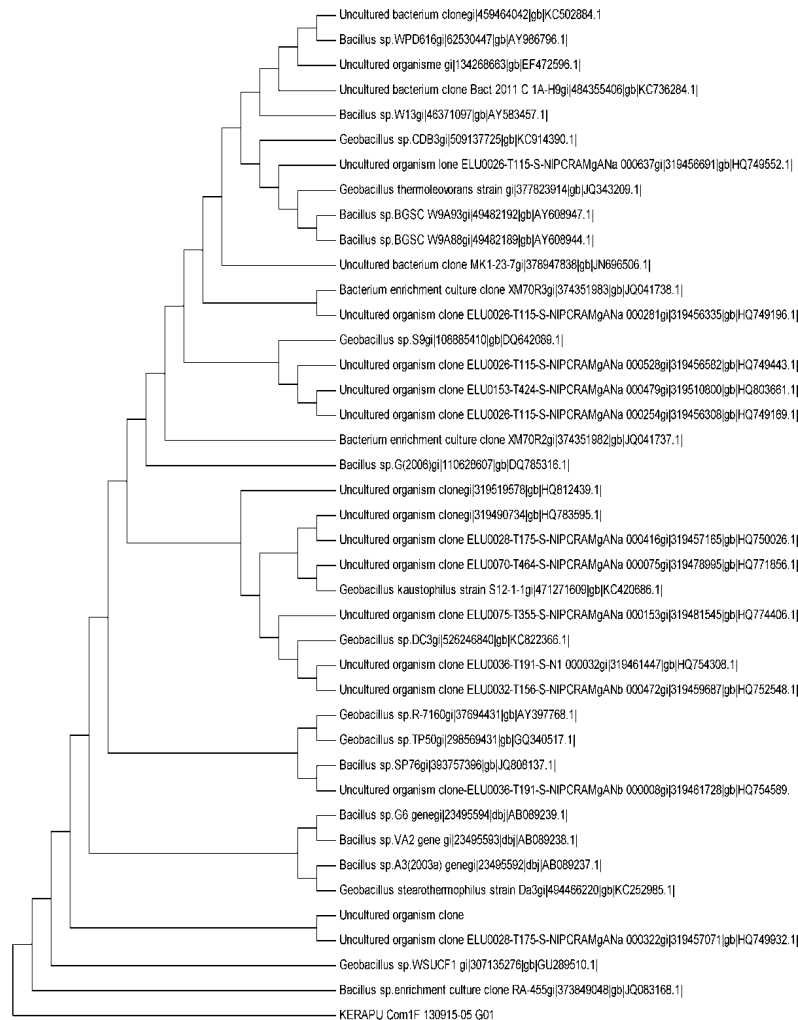


Figure 6. The phylogenetic tree revealed no evolutionary relationship between the isolated bacteria (grouper bacteria)

Several studies have employed similar approaches to assess the evolutionary relatedness of isolated bacteria and compare them with other bacteria exhibiting similarities. Investigated the evolutionary relationships of bacteria isolated from marine environments using phylogenetic analysis demonstrated that certain isolated bacterial strains showed distinct phylogenetic divergence from other bacteria, suggesting the presence of novel or uncultured bacterial lineages[16], [27], [37]. Another study conducted a phylogenetic analysis of bacterial isolates from soil samples. They observed that some of the isolated bacteria did not exhibit an evolutionary relationship with the closely related bacteria, indicating the potential existence of uncharacterized bacterial taxa in the environment[38], [39].

These studies highlight the importance of conducting phylogenetic analysis to unravel the evolutionary relationships between isolated bacteria and related species. They also emphasize the need for additional purification steps and more precise genetic analyses, such as the analysis of specific genetic markers like the 16S rRNA gene, to ensure accurate and reliable results in determining the origin and evolutionary affiliations of isolated bacteria.

CONCLUSION

This study successfully isolated formaldehyde-producing bacteria from the gastrointestinal tract of tiger grouper. These bacteria play a crucial role in the conversion of TMAO into formaldehyde. The identification and characterization of these bacteria are essential for understanding their contribution to various biological processes and environmental systems. The findings highlight the presence of previously uncharacterized or uncultured bacteria, as they did not demonstrate an evolutionary relationship with other bacteria displaying similarities. Further purification and analysis of the 16S rRNA gene are required to obtain more accurate and reliable results. These bacteria hold significant implications in food safety, scientific research, medical diagnostics, and industrial applications. Future investigations should focus on elucidating the enzymatic pathways and genetic mechanisms underlying formaldehyde production in these specific bacteria, which will provide valuable insights into their unique characteristics and potential applications.

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