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Antibacterial Activity of Probiotics from *Naniura* Made with Nile Tilapia (*Oreochromis niloticus*) against *Salmonella typhi*

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Abstract: Microbial contamination in foodstuffs, particularly by pathogenic bacteria such as *Salmonella typhi*, is one of the leading causes of foodborne diseases, posing a serious threat to public health. The use of antibiotics to control infections has led to an increase in antibiotic resistance cases, thereby necessitating safer and more sustainable alternatives for controlling pathogenic bacteria. One potential approach is the utilization of natural probiotics derived from traditional fermented foods. *Naniura*, a traditional dish from North Sumatra made from raw freshwater fish without any heating process, is believed to contain lactic acid bacteria (LAB) that function as probiotics. This study aims to evaluate the antibacterial activity of probiotics derived from *naniura* made with Nile tilapia (*Oreochromis niloticus*) against *Salmonella typhi*. Nile tilapia was selected due to its high protein content and meat structure with fewer bones compared to common carp. The fermentation of *naniura* was carried out for 10 hours to optimize the growth of probiotic microorganisms. Probiotic isolation was followed by macroscopic, microscopic, and biochemical characterizations using TSIA, citrate, motility, indole, ornithine, MRVP, catalase, amylolytic, and proteolytic activity tests. The antibacterial activity was evaluated using the disk diffusion method against *Salmonella typhi*. The results showed that the isolated probiotics exhibited morphological and physiological characteristics indicative of the genus *Enterococcus*, and the diameter of the inhibition zone produced is categorised as strong, with an average inhibition zone of 12.8 mm ± 0.36 against *Salmonella typhi*. This study demonstrates that *naniura* made from Nile tilapia has the potential to serve as a natural source of effective antibacterial probiotics.

Keywords: Antibacterial Activity; *Naniura*; Nile tilapia; Probiotics; *Salmonella typhi*.

Introduction

Microbial contamination in food is a serious issue that can occur through direct or indirect exposure to various sources such as soil, air, water, dust, as well as the digestive and respiratory tracts of humans or animals. One of the consequences of such contamination is foodborne disease, which refers to illnesses transmitted through food contaminated with pathogenic microorganisms. One common foodborne disease is typhoid fever, an acute infection of the gastrointestinal tract caused by the bacterium *Salmonella typhi* [1]. Typhoid fever remains a global health threat, particularly in developing countries with limited sanitation infrastructure [2]. According to data from the World Health Organization (WHO), there are approximately 9 million new cases of typhoid fever worldwide each year, with over 110,000 deaths reported annually [3]. In Indonesia, typhoid fever is considered an endemic disease with a high prevalence, ranging from 500 to 1,200 cases per 100,000 population each year [3].

The use of antibiotics has long been the primary method for preventing and treating bacterial infections. However, improper or excessive use of antibiotics can lead to bacterial resistance, which has become a major challenge in the field of healthcare. Therefore, there is a need for safe and sustainable alternative solutions to control pathogenic bacteria, one of which is the utilization of probiotics derived from traditional fermented foods [4]. Probiotics are live microorganisms that provide health benefits to the host, primarily through their ability to produce antibacterial compounds. These compounds act through various mechanisms, such as disrupting bacterial cell walls, altering cytoplasmic membrane permeability, inhibiting enzymes, and interfering with protein and nucleic acid synthesis [5]. Several antibacterial compounds produced by probiotic microorganisms include organic acids such as acetic and lactic acids, diacetyl, hydrogen peroxide, and bacteriocins in the form of polypeptides or proteins [6].

Naniura is a traditional dish from North Sumatra that has a unique characteristic in that it is not subjected to cooking processes such as boiling, frying, or steaming [7]. This dish is made from fresh fish marinated in a mixture of jungga lime and spices, where the acidic marinade helps reduce fishy odor and imparts a distinctive flavor [8]. This fermentation-like process potentially supports the growth of lactic acid bacteria (LAB), particularly because the main ingredient is freshwater fish, which is known to harbor microorganisms such as *Lactobacillus* and *Streptococcus* [9]. A previous study by Nasri [10], demonstrated that *naniura* made from common carp could produce probiotic bacteria with *Lactobacillus* characteristics and exhibited antibacterial activity against *Salmonella typhi*, with an inhibition zone diameter of 12.9 mm. However, scientific studies on the probiotic potential of *naniura* remain limited.

In this study, fermentation was carried out for 10 hours, which is considered the optimal duration to support the growth of probiotic microorganisms and the production of bioactive metabolites [11]. Other studies have shown that

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fermented products such as moringa leaf yogurt and kimchi exhibit significant antibacterial activity against pathogens such as *Escherichia coli* and *Salmonella typhimurium* [12], [13]. In general, the primary ingredient used in *naniura* is common carp. However, this fish has the drawback of containing numerous fine bones, which can reduce consumer comfort during consumption [14]. A potential alternative is Nile tilapia, which has a similar texture and flavor but offers better nutritional value and fewer bones. According to nutritional data, Nile tilapia contains 43.76% protein, 7.01% fat, 6.80% ash, and 4.28% moisture per 100 grams—figures that are superior to those of catfish [15].

Based on these considerations, further research is needed to evaluate the potential antibacterial activity of *naniura* made from Nile tilapia against *Salmonella typhi*. This study is expected to serve as an initial step in the development of functional foods based on traditional fermentation as an alternative solution to antibiotic resistance and to support more natural and sustainable treatment of typhoid fever. This study is the first to evaluate the probiotic potential of *naniura* made from Nile tilapia using comprehensive microbiological testing.

Research Methods

The apparatus utilized in this study comprised an incubator, autoclave, dropper pipettes, Erlenmeyer flasks, Petri dishes, Bunsen burner, beakers, blender, measuring cup, laminar air flow (LAF) cabinet, analytical balance, test tubes along with a test tube rack, sterile gloves, scissors and tweezers, inoculating loop, refrigerator, hot plate stirrer, microscope slides and cover glasses, compound microscope, digital caliper, and micropipettes.

The materials used in this study included *naniura* samples made from Nile tilapia (*Oreochromis niloticus*), specifically the fish fillet portion; *Salmonella typhi* bacterial isolate; distilled water (aquadest); MRSA (de Man Rogosa Sharpe Agar); starch; SMA (Skim Milk Agar); Simmons Citrate Agar (SCA); MIO medium (Motility Indole Ornithine); MRVP medium (Methyl Red and Voges–Proskauer); Nutrient Agar (NA); Mueller Hinton Agar (MHA); Triple Sugar Iron Agar (TSIA); 3% H_2O_2; 40% KOH; 5% α-naphthol; methyl red; Kovac's reagent; crystal violet; Lugol's iodine; 95% ethanol; 70% alcohol (for sterilization); paper discs; immersion oil; 1% sulfuric acid (H_2SO_4); 1% barium chloride ($BaCl_2$); 1% calcium carbonate ($CaCO_3$); physiological saline (NaCl); ciprofloxacin; cotton; aluminum foil; and labeling paper.

Preparation of Nile Tilapia-Based *Naniura*

A total of 20 g of Nile tilapia fillet was placed in a closed container and evenly soaked with the juice of *jeruk jungga* (a local citrus fruit), then left to stand for 1 hour. This step serves as the marination process, which aims to soften the fish bones and tenderize the flesh without the application of heat. Subsequently, a mixture of spices consisting of 9 g shallots, 4 g garlic, 5 g candlenuts, 15 g *kecombrang* (torch ginger flower), 2 g ginger, 4 g turmeric, and 1 g *andaliman* (a type of Szechuan pepper) was dry-roasted until aromatic. The roasted spices were then ground together with 1 g of chili. Once the mixture reached a smooth consistency, it was evenly applied to the surface of the Nile tilapia fillet, which was then left to ferment for 10 hours [16].

Probiotic Bacteria Isolation from Nile Tilapia-Based *Naniura*

One milliliter of the *naniura* sample in the form of extract was added to a tube containing 9 mL of 0.9% physiological NaCl solution and homogenized. Subsequently, serial dilutions were performed to achieve dilutions ranging from 10^{-1} to 10^{-7}. MRS agar medium, supplemented with 1% $CaCO_3$, was poured into Petri dishes using the pour plate method and allowed to solidify. From the last three dilution series, 1 mL of the sample was taken from each and placed into Petri dishes, then incubated at 37°C for 48 hours [17].

Colonies that formed clear zones on MRS agar medium (suspected to be lactic acid bacteria) were picked using an inoculating loop and re-inoculated onto MRS agar medium using the streak plate method. Incubation was continued at 37°C for 48 hours. The streak plate procedure was repeated 3 times until uniform and well-separated colonies were obtained. The pure isolates obtained were then stored on slant MRS agar for further analysis [17].

Morphological Characterization of Probiotic Isolates from Nile Tilapia-Based *Naniura*

The characterization process for determining the morphology of probiotic isolates was conducted using two methods: macroscopic and microscopic. The macroscopic morphology of the probiotic isolates was visually observed, including the shape, margin, color, and elevation of the bacterial colonies [18].

The microscopic examination was performed using the Gram staining method. The procedure began by placing two drops of sterile distilled water on a glass slide, followed by the addition of the probiotic bacterial isolate sample, which was then spread aseptically using an inoculating needle. The preparation was then dried by passing it over a Bunsen burner flame until completely dry. The staining process commenced with the addition of crystal violet stain (2-3 drops) and allowed to sit for 1 minute, followed by rinsing with distilled water. The preparation was then treated with iodine solution (1-2 drops), allowed to sit for 1 minute, and rinsed again. The decolorization step was performed by adding 96% alcohol for 30 seconds, followed by washing with distilled water. The final staining was done by adding safranin (2-3 drops), allowing it to sit for 1 minute, and then rinsing and drying again. The dried preparation was observed under a microscope using immersion oil. Gram-positive bacteria were identified by their purple color, while Gram-negative bacteria appeared red [19].

Biochemical Characterization of Probiotic Isolates from Nile Tilapia-Based *Naniura*

The biochemical tests conducted included the Triple Sugar Iron Agar (TSIA) test, citrate test, indole motility ornithine test, MRVP test, catalase test, and tests for amylolytic and proteolytic activity.

TSIA test

The TSIA test was conducted to evaluate the capacity of bacteria to metabolize glucose, lactose, and sucrose, in addition to examining gas and H_2S production. A pure bacterial isolate was inoculated into TSIA medium by stabbing the butt and streaking the slant in a zig-zag manner, followed by incubation at 35ºC for a duration of 24 hours. Observations were made based on alterations in the medium's color: a yellow hue in the butt signified glucose fermentation, a yellow hue in both the butt and slant indicated fermentation of glucose, lactose, and sucrose, whereas a red hue showed that fermentation did not occur. The presence of black precipitate confirmed H_2S production, and the formation of gas bubbles indicated gas production [20].

Citrate test

The citrate test was performed to assess whether the bacteria could utilize citrate as their sole carbon source. A pure bacterial isolate was inoculated once by streaking in a zig-zag pattern across the surface of Simmon's Citrate agar, followed by incubation at 29°C for 24 hours. A positive result was indicated by a color change of the medium from green to blue, while no color change (remaining green) indicated a negative result [21].

MIO test

The MIO test was carried out to evaluate the bacterial capabilities for motility, indole production, and ornithine decarboxylation. A pure bacterial isolate was inoculated by stabbing straight down into the center of the MIO medium, nearly reaching the bottom of the tube, and then incubated at 37°C for 24 hours. Motility was considered positive if bacterial growth diffused away from the stab line. A positive result for ornithine decarboxylase activity was indicated by a color shift to purple or grayish-purple, while a yellow color signified a negative outcome. Indole production was confirmed by the appearance of a red ring upon the addition of Kovac's reagent [22], [23].

MRVP test

The MRVP test was performed to identify the bacterial glucose fermentation pathway, distinguishing between the production of strong acids (Methyl Red test) and neutral or non-acidic end products (Voges-Proskauer test). A pure bacterial isolate was inoculated into MRVP broth and incubated at 37°C for 24 hours. After incubation, the culture was split into two separate tubes. For the MR test, three drops of methyl red indicator were added, with a red color indicating a positive result. For the VP test, three drops of 40% KOH and six drops of 5% α-naphthol were added, followed by shaking for 30 seconds; the appearance of a pink color signified a positive reaction [21].

Catalase test

The catalase test was conducted to determine whether the bacteria produced the catalase enzyme. A pure bacterial isolate was introduced into a drop of 3% hydrogen peroxide (H_2O_2) placed on a glass slide. The presence of gas bubbles, resulting from the enzymatic breakdown of H_2O_2, indicated a positive reaction. The absence of bubble formation was interpreted as a negative result [24].

Amylolytic activity test

This test was carried out to assess the bacterial ability to hydrolyze starch by producing amylase enzymes. A pure bacterial isolate was streaked onto Starch Agar and incubated at 35°C for 24 hours. After incubation, iodine solution was applied to the surface of the medium. A clear zone surrounding the bacterial growth indicated a positive result, demonstrating starch hydrolysis activity [25].

Proteolytic activity test

This test was performed to evaluate the bacterial ability to produce protease enzymes capable of hydrolyzing proteins. A loopful of pure bacterial isolate was streaked onto Skim Milk Agar (SMA) and incubated at 35°C for 24 hours. A clear zone surrounding the bacterial colony indicated a positive result, reflecting proteolytic activity [25].

Evaluation of the Antibacterial Activity of *Naniura*-Derived Probiotics Against *Salmonella typhi*

The antibacterial activity was assessed using the disk diffusion technique. The test bacterial suspension was prepared by adjusting its turbidity to the equivalent of the 0.5 McFarland standard. A sterile cotton swab was dipped into the suspension, and the excess liquid was removed by pressing it against the inner wall of the tube. The swab was then used to evenly spread the bacteria across the surface of solidified Mueller Hinton Agar (MHA) in three directions. The plate was left undisturbed for 5 minutes to allow the surface to dry. Sterile paper disks (5 mm in diameter) were soaked in the *naniura* extract, sterile distilled water (serving as a negative control), and a 5% ciprofloxacin solution (as a positive control). Using sterile forceps, the disks were placed on the inoculated MHA surface with gentle pressure. Within 15 minutes of disk placement, the Petri dishes were incubated in an inverted position at 37°C for 24 hours to prevent condensation from affecting the medium [26]. Following incubation, the presence of inhibition zones (clear areas) surrounding the disks was observed. The diameters of these zones were measured in both vertical and horizontal directions, and the diameter of the disk was subtracted to determine the effective inhibition zone diameter [27].

Results And Discussion

Isolation of Probiotic Bacteria from *Naniura* of Nile Tilapia

Bacterial isolation was performed using MRS agar, a selective medium commonly used for the cultivation of lactic acid-producing or potentially probiotic bacteria [28]. A total of 1 mL of sample from each dilution (10^{-1} to 10^{-7}) was inoculated and incubated at 37°C for 48 hours. This temperature was chosen because it falls within the optimal

growth range for probiotic bacteria, which is 37–41°C [29]. Temperatures that are too low may inhibit bacterial growth, while higher temperatures may pose a risk of killing bacterial cells [30], [31].

After incubation, colonies that formed clear zones were selected as probiotic candidates. These zones indicate the formation of calcium lactate resulting from the reaction between lactic acid and $CaCO_3$ [32]. Isolates were selected from the 10^{-6} and 10^{-7} dilutions to obtain single colonies and avoid contamination, as higher dilutions result in fewer microbial colonies [33]. The selected colonies were then purified using the quadrant streak method, repeated four times to obtain pure isolates. The pure isolates were subsequently cultured on slanted MRS agar for further identification.

Morphological Characterization of Probiotic Isolates from *Naniura* of Nile Tilapia

Morphological characterization was conducted both macroscopically and microscopically. Macroscopic characterization of bacterial morphology was based on visible features, including colony shape, color, elevation (surface profile), and edge or margin characteristics. The pure isolates obtained showed macroscopic morphology with irregular colony shape, white color, flat-raised elevation, and undulate (wavy) margins. These characteristics are consistent with the colony morphology of lactic acid bacteria (LAB) as described by Apriyani [34], which include irregular shape, raised elevation, white color, and wavy margins.

Microscopic characterization was carried out using Gram staining, followed by observation under a microscope at 100x magnification. The observations showed that the obtained pure isolates were Gram-positive with a coccus (spherical) shape, indicated by a purple coloration due to the retention of crystal violet, which is not dissolved during alcohol rinsing [35]. Gram-positive bacteria have thick cell walls composed of up to 90% peptidoglycan and low lipid content, which allows the crystal violet stain to remain strongly bound [36]. Additionally, the presence of teichoic acid in the cell wall contributes to the durability and structural integrity of the cell, making Gram-positive bacteria commonly used as probiotics [37]. These findings are consistent with the characteristics of lactic acid bacteria (LAB), which are generally rod- or coccus-shaped [32].

Biochemical Characterization of Probiotic Isolates from *Naniura* of Nile Tilapia

Biochemical testing is an identification method that relies on the observation of bacterial physiological characteristics [38]. The results of these tests may vary among isolates, indicating differences in physiological traits influenced by the enzymatic activity of each isolate in utilizing reagents during biochemical processes.

The TSIA test results showed a yellow coloration in both the slant and butt of the medium, indicating an A/A (acid/acid) reaction. This suggests that the isolate is capable of fermenting all three sugars: glucose, lactose, and sucrose. The absence of gas bubbles and black precipitate indicates that the isolate does not produce gas or hydrogen sulfide (H_2S) [36]. The shift in color from red to yellow is caused by a pH decrease resulting from acid production during the fermentation process [39]. The citrate test yielded a negative result, as indicated by the absence of a color change in the medium. This suggests that the isolate does not produce the enzyme citrate permease and is therefore unable to utilize citrate as a carbon source in its metabolism [40]. The motility test showed a positive result, indicated by the growth of bacteria spreading from the stab line, meaning that the obtained isolate is motile or has flagella [41]. The color change of the medium to a grayish-purple indicates that the isolate is capable of degrading ornithine through decarboxylase enzyme activity [42]. Similarly, the indole test showed a negative result, indicated by the lack of color change after the addition of Kovac's reagent, suggesting that the isolate does not possess the enzyme tryptophanase [43]. The MR test produced a positive result, as evidenced by the medium turning red after the addition of methyl red reagent. This indicates that the isolate is capable of producing acidic compounds, such as lactic acid, formic acid, and acetic acid, which lower the pH of the medium [44]. The VP test showed a negative result, indicated by the absence of pink color formation after the addition of the reagent. This indicates that the isolate does not produce acetoin but rather predominantly generates strong acidic compounds during the fermentation of glucose [45], [46]. The catalase test yielded a negative result, as no bubble formation was observed upon the addition of H_2O_2. This suggests that the isolate is a homofermentative anaerobic bacterium that does not produce the catalase enzyme [47], [48].

The amylolytic activity test showed a positive result, as evidenced by the appearance of a clear zone surrounding the colony after the addition of iodine. This indicates that the bacteria are capable of hydrolyzing starch [49]. This ability suggests that the isolate produces the enzyme amylase as an effort to obtain energy sources from starch [50], [51]. The proteolytic activity test yielded a positive result, indicated by the formation of a clear zone around the colony. This clear zone reflects the activity of protease enzymes that hydrolyze casein into amino acids [52]. Protease activity is affected by various factors such as temperature, pH levels, and the concentrations of both the enzyme and its substrate [53].

Table 1. Results of biochemical tests of probiotic isolates from *naniura* of nile tilapia

Characteristics	Results
TSIA test	A/A
Citrate test	-
MR test	+
VP test	-
Catalase test	-
Motility, Indole, Ornithine test	M, -, +
Amylolytic Activity Test	+
Proteolytic Activity Test	+

Note: A/A = Acid/acid, M = motile, (+) = positive reaction, (–) = negative reaction

Identification of the Genus of Probiotic Bacteria

The bacterial isolate obtained showed characteristics consistent with bacteria from the genus *Enterococcus*. According to Cowan and Steel's Manual for the Identification of Medical Bacteria, *Enterococcus* species are Gram-positive cocci, with some species being motile,

catalase-negative, and facultatively anaerobic [54]. *Enterococcus* is classified as a lactic acid bacterium found in soil, surface water, and even marine environments [55]. Phylogenetically, *Enterococcus* belongs to the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, and family *Enterococcaceae* [56].

Antibacterial Activity of Probiotic from *Naniura* of Nile Tilapia against *Salmonella typhi*

The antibacterial activity test was performed using the disk diffusion method with 5 mm diameter paper disks. Observations were made by examining the clear zones around the disks as indicators of antibacterial activity. Sterile distilled water was used as a negative control, and 5% ciprofloxacin served as the positive control. Ciprofloxacin is a fluoroquinolone-class antibiotic effective against Gram-negative bacteria, including *Salmonella typhi*, with reported inhibition zones ranging from 26–33 mm [57]. In this study, ciprofloxacin produced a wide inhibition zone, indicating its effectiveness as both a bactericidal and bacteriostatic agent [58]. while sterile distilled water showed no inhibition zone due to its neutral properties [59]. The antibacterial activity is illustrated in Figure 1.

Figure 1. Antibacterial activity of *naniura* probiotics and control against *S. typhi*

The inhibition zone diameters for the *naniura* treatment, positive control (*ciprofloxacin*), and negative control (sterile distilled water) against *Salmonella typhi* are presented in Table 2.

Table 2. Inhibition zone diameter of *naniura* and control of *S.typhi*

Treatment	Diameter (mm) ± SD (n=3)
Naniura Probiotics	12.8 ± 0.36
Ciprofloxacin (control +)	28.1 ± 0.17
Sterile distilled water (control -)	0

Based on Table 2, it shows that the probiotic from naniura made from Nile tilapia can inhibit the growth of *Salmonella typhi* with an inhibition zone diameter ranging from 10 to 20 mm. According to the classification by Rahayuningsih et al. [60], this value falls into the strong inhibition category, although according to CLSI (Clinical and Laboratory Standards Institute) standards, it is still classified as resistant (≤14 mm).

According to the Clinical and Laboratory Standards Institute (CLSI), the interpretation of inhibition zones against Gram-negative bacteria such as *Salmonella* using standard antibiotics is categorized as susceptible (≥21 mm), intermediate (16–20 mm), and resistant (≤15 mm), depending on the type of antibiotic. Although *naniura* extract is not a synthetic antibiotic and cannot be directly compared, the 12.8 mm ± 0.36 inhibition zone indicates moderate antibacterial activity. This suggests that the *Enterococcus* isolate from *naniura* has potential as a natural probiotic agent capable of inhibiting enteric pathogens [61]. However, the disk diffusion method has limitations, such as its inability to determine the Minimum Inhibitory Concentration (MIC), distinguish between bacteriostatic and bactericidal effects, or provide time–kill dynamics [62].

This antibacterial potential is suspected to originate from LAB that has probiotic potential with the genus *Enterococcus* identified from the isolate. In addition to producing organic acids, *Enterococcus* is also known to be capable of producing bacteriocins, which are antimicrobial extracellular protein or peptide compounds [63]. Bacteriocins work by forming pores in the membranes of pathogenic cells, causing leakage of cell contents and disrupting homeostasis, ultimately inhibiting growth and leading to the death of pathogenic cells [64], [65], [66]. A similar study by Artha [67], showed that *Enterococcus faecium* can inhibit *Listeria monocytogenes* through the same mechanism.

Conclusion

The probiotic bacterial isolate from *naniura* of Nile tilapia (*Oreochromis niloticus*) has characteristics of white colonies, Gram-positive cocci, and is actively fermenting glucose, lactose, and sucrose. The isolate showed positive results in the MR test, ornithine, motility, amylolytic, and proteolytic tests, while being negative in the citrate, VP, indole, and catalase tests. The probiotic from *naniura* of Nile tilapia effectively inhibits *Salmonella typhi*, as indicated by a strong inhibition zone in the disk diffusion test, demonstrating its potential as a natural antibacterial agent. This finding supports the development of functional foods based on traditional fermentation and highlights the potential role of indigenous probiotic sources in reducing antibiotic resistance and improving public health through natural dietary interventions.

Author's Contribution

Agis Ayu Cahyani: was responsible for conducting the research, data collection, result analysis and manuscript drafting. Rudiana Agustini: served as the corresponding author, providing academic guidanceand reviewing and refining the article draft.

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References

[1] A. I. Maharani, A. F. Sari, and L. Advinda, "Kualitas Mikrobiologi Daging Sapi dari Swalayan─Mini Review," *Prosiding Semnas Bio*, vol. 1, no. 1, pp. 624–634, 2021.

[2] S. Contini, "Typhoid intestinal perforation in developing countries: Still unavoidable deaths?," *World J Gastroenterol*, vol. 23, no. 11, pp. 1925–1931, Mar. 2017, doi: 10.3748/wjg.v23.i11.1925.

[3] O. N. Laila, Khambali, and I. Sulistio, "Perilaku, Sanitasi Lingkungan Rumah dan Kejadian Demam Tifoid," *Jurnal Penelitian Kesehatan Suara Forikes*, vol. 13, no. 2, pp. 525–529, 2022, doi: 10.33846/sf13247.

[4] A. S. Marsudi, W. I. Wiyono, and D. A. Mpila, "Tingkat Pengetahuan dan Perilaku Masyarakat terhadap Penggunaan Antibiotik di Beberapa Apotek di Kota Ternate," *Pharmacy Medical Journal*, vol. 4, no. 2, pp. 54–61, 2021.

[5] H. B. Aviany and S. Pujiyanto, "Analisis Efektivitas Probiotik di dalam Produk Kecantikan sebagai Antibakteri terhadap Bakteri *Staphylococcus epidermidis*," *Berkala Bioteknologi*, vol. 3, no. 2, pp. 24–30, 2020.

[6] S. J. Nurhamidin, D. S. Wewengkang, and E. J. Suoth, "Uji Aktivitas Ekstrak dan Fraksi Organisme Laut Spons *Aaptos aaptos* terhadap Bakteri *Escherichia coli* dan *Staphylococcus aureus*," *PHARMACON*, vol. 11, no. 1, pp. 1285–1291, 2022.

[7] S. N. Cristy and L. Chunliu, "Makanan Tradisional Batak Toba: Kajian Metabahasa Semantik Alami," *Jurnal Ilmiah Pendidikan Bahasa, Sastra Indonesia dan Daerah*, vol. 14, no. 1, pp. 411–425, 2024.

[8] O. J. Tarigan, "Pengaruh Jenis Asam dan Lama Marinasi Terhadap Karakteristik Sensoris, Mikrobiologis, dan Kimia Naniura Ikan Nila (*Oreochromis niloticus*)," *Jurnal Teknologi Hasil Perikanan*, vol. 5, no. 2, pp. 112–122, 2016.

[9] Aloysius, "Aktivitas Antimikroba Bakteri Asam Laktat yang Diisolasi dari Makanan Tradisional Fermentasi Khas Batak 'Naniura,'" *Jurnal Biologi Lingkungan, Industri, Kesehatan*, vol. 6, no. 1, pp. 8–15, 2019.

[10] Nasri, "Uji Aktivitas Antibakteri Probiotik dari Ikan Naniura Hasil Fermentasi Ikan Mas (*Cyprinus carpio L.*) terhadap *Salmonella typhi*," Universitas Sumatera Utara, Medan, 2019.

[11] Z. Gumanti, A. P. Salsabila, and M. E. Sihombing, "Pengaruh Lama Fermentasi terhadap Mutu Organoleptik pada Proses Pembuatan Kombucha Sari Kulit Buah Naga Merah (*Hylocereus polyrhizus*)," *Jurnal Pengolahan Pangan*, vol. 8, no. 1, pp. 25–32, 2023.

[12] I. Ismail, R. Riski, and N. K. Salsabila, "Isolasi, Formulasi, dan Uji Efektivitas Antibakteri Granul Liofilisat Bakteri Asam Laktat Asal Kimchi terhadap Bakteri Penyebab Diare," *Pharmaceutical Journal of Indonesia*, vol. 19, no. 01, pp. 171–183, 2022.

[13] R. Setia and E. D. Wijayanti, "Aktivitas Antibakteri Yoghurt Daun Kelor (*Moringa oleifera*) dan Lidah Buaya (*Aloe vera*) terhadap *Escherichia coli*," Akademi Farmasi Putera Indonesia Malang, Malang, 2019.

[14] J. Sinulingga, "Kajian Masakan Khas Batak Toba Naniura dari Sudut Pandang Semiotik Sosial," *Jurnal Pendidikan Tambusai*, vol. 8, no. 2, pp. 24676–24684, 2024.

[15] R. A. Yulianti, "Analisa Kualitas Air terhadap Pola Pertumbuhan Ikan Nila di Kolam Semi Beton dan Kolam Tanah pada Desa Sumbersekar Kecamatan Dau, Kabupaten Malang," Universitas Brawijaya, Malang, 2015.

[16] J. R. Melba, "Pelestarian Dekke Naniura pada Upacara Adat Batak di Pulau Samosir, Sumatera Utara," Universitas Pendidikan Indonesia, Bandung , 2023.

[17] S. Bulu, M. E. S. Ledo, and A. D. N. Rupidara, "Identifikasi Bakteri Asam Laktat pada Nira Segar Lontar (*Borassus flabellifer Linn*)," *Jambura Edu Biosfer Journal*, vol. 1, no. 2, pp. 47–52, 2019.

[18] Zuraidah, D. Wahyuni, and E. Astuty, "Karakteristik Morfologi dan Uji Aktivitas Bakteri Termofilik dari Kawasan Wisata Ie Seuum (Air Panas)," *Jurnal Ilmu Alam dan Lingkungan*, vol. 11, no. 2, pp. 40–47, 2020.

[19] Z. Z. N. Amaliah, S. Bahri, and P. Amelia, "Isolasi dan Karakterisasi Bakteri Asam Laktat dari limbah Cair Rendaman Kacang Kedelai," *Jurnal Fitofarmaka Indonesia*, vol. 5, no. 1, pp. 253–257, 2018.

[20] P. Dahal, "TSIA Test: Principle, Media, Procedure, Results, Uses," https://microbenotes.com/triple-sugar-iron-agar-tsia-test/.

[21] S. S. B. Ginting, D. Suryanto, and D. Desrita, "Isolasi dan Karakterisasi Bakteri Potensial Probiotik pada Saluran Pencernaan Ikan Bandeng (*Chanos chanos*)," *Acta Aquatica: Aquatic Sciences Journal*, vol. 5, no. 1, pp. 23–29, Apr. 2018, doi: 10.29103/aa.v5i1.390.

[22] E. Karunia, R. Kurniatuhadi, and A. Hepi Yanti, "Karakterisasi Bakteri *Bacillus sp.* (kode NrLtF5) yang Diisolasi dari Usus Cacing Nipah (*Namalycastis rhodochorde*)," *Protobiont*, vol. 10, no. 3, pp. 69–73, 2021.

[23] A. Sulistiyono and E. Mutiara, "Pengujian Bakteri Patogen pada Ikan Hias di Stasiun Karantina Ikan Pengendalian Mutu dan Keamanan Hasil Perikanan Palembang," *Sriwijaya Bioscientia*, vol. 3, no. 3, pp. 1–9, Jan. 2023, doi: 10.24233/sribios.3.3.2022.358.

[24] G. Fallo, L. Pardosi, and A. Y. Boluk, "Seleksi Bakteri Pelarut Fosfat dari Rhizosfer Tanaman Cabai Rawit (*Capsicum annuum L.*) di Kabupaten Timor Tengah Utara," *BIOSENSE*, vol. 5, no. 1, pp. 24–33, 2022.

[25] A. Andriyanto and E. Yulianti, "Identifikasi Bakteri Probiotik pada Saluran Pencernaan Ikan Semah (*Tor Sp.*)," *BIOEDUSAINS: Jurnal Pendidikan Biologi dan Sains*, vol. 3, no. 2, pp. 120–131, Dec. 2020, doi: 10.31539/bioedusains.v3i2.1804.

[26] Y. R. Rishliani, "Uji Aktivitas Antibakteri Ekstrak Etanol Daun Nanas (*Ananas comosus L. Merr.*) terhadap *Propionibacterium acnes*," Universitas Jambi, Jambi, 2022.

[27] S. S. Katili, D. S. Wewengkang, and H. Rotinsulu, "Uji Aktivitas Antimikroba Ekstrak Etanol Organisme Laut Spons *Ianthella basta* terhadap Beberapa Mikroba Patogen," *PHARMACON Jurnal Ilmiah Farmasi-UNSRAT*, vol. 9, no. 1, pp. 100–107, 2020.

[28] N. P. Ningsih, R. Sari, and P. Apridamayanti, "Optimasi Aktivitas Bakteriosin yang Dihasilkan Oleh *Lactobacillus brevis* dari Es Pisang Ijo," *Jurnal Pendidikan Informatika dan Sains*, vol. 7, no. 2, pp. 2407–1536, 2018.

[29] R. Ihsan and D. Cakrawati, "Penentuan Umur Simpan Yoghurt Sinbiotik dengan Penambahan Tepung Gembolo Modifikasi Fisik," *EDUFORTECH*, vol. 2, no. 1, pp. 1–6, 2017.

[30] W. Liszkowska and J. Berlowska, "Yeast fermentation at low temperatures: Adaptation to changing environmental conditions and formation of volatile compounds," *Molecules*, vol. 26, no. 4, pp. 1–21, Feb. 2021, doi: 10.3390/molecules26041035.

[31] J. A. Rorong and W. F. Wilar, "Keracunan Makanan Oleh Mikroba," *Techno Science Journal*, vol. 2, no. 2, pp. 47–60, 2020.

[32] R. Rumaisha, O. Suzanti Betha, and H. Aldrat, "Isolasi dan Karakterisasi Bakteri Asam Laktat dari Kefir Susu Kambing Saanen (*Capra aegagrus Hircus*)," *Pharmaceutical and Biomedical Sciences Journal*, vol. 2, no. 2, pp. 79–86, 2020.

[33] Rosmania and Yuniar, "Pengaruh Waktu Penyimpanan Inokulum *Escherichia coli* dan *Staphilococcus aureus* pada Suhu Dingin terhadap Jumlah Sel Bakteri di Laboratorium Mikrobiologi," *Jurnal Penelitian Sains*, vol. 23, no. 3, pp. 117–124, 2021, [Online]. Available: http://ejurnal.mipa.unsri.ac.id/index.php/jps/index

[34] R. Apriyani, "Isolasi dan Uji Potensi Bakteri Asam Laktat Asal Buah Nipah (*Nypa fruticans*) dalam Memfermentasi Kopi Gayo," Universitas Islam Negeri Ar-Raniry, Banda Aceh, 2020.

[35] M. N. Hamidah, L. Rianingsih, and Romadhon, "Aktivitas Antibakteri Isolat Bakteri Asam Laktat dari Peda dengan Jenis Ikan Berbeda terhadap *E. coli* dan *S. aureus*," *Jurnal Ilmu dan Teknologi Perikanan*, vol. 1, no. 2, pp. 11–21, 2019.

[36] P. S. Martati, "Pengaruh Liofilisasi Bakteri *Staphylococcus aureus* ATCC 25923 dengan Serum Kuda Sebagai Lioprotektan yang Disimpan Selama Dua Bulan pada Suhu 4°C terhadap Viabilitas, Morfologi dan Biokimia," Poltekkes Kemenkes Yogyakarta, Yogyakarta, 2023.

[37] K. A. Istiadi, *Mikrobiologi dan Virologi* . Medan: Yayasan Kita Menulis, 2024.

[38] D. P. Suherman, "Uji Viabilitas dan Pengamatan Morfologi Liofilisat Bakteri *Pseudomonas aeruginosa* yang disimpan Selama Dua Bulan pada Suhu -20°C," Poltekkes Kemenkes Yogyakarta, Yogyakarta, 2022.

[39] N. R. Lailiya, "Isolasi dan Identifikasi Bakteri Toleran terhadap Logam Berat Pb pada Air dan Sedimen di Sungai Porong Sidoarjo Jawa Timur," Universitas Islam Negeri Sunan Ampel, Surabaya, 2021.

[40] N. S. Rohmah, "Isolasi dan Identifikasi Bakteri yang Berpotensi Sebagai Agen Bioremediasi Timbal (Pb) dari Lumpur Lapinđo," Universitas Islam Negeri Maulana Malik Ibrahim, Malang, 2017.

[41] Nurmila, "Identifikasi Bakteri pada Perairan Danau Manuk Bungkul Kecamatan Sembakung Kabupaten Nunukan," Universitas Borneo, Tarakan, 2020.

[42] A. A. Nopiosi, T. R. Ferasyi, and M. Abrar, "Pemeriksaan Jumlah Cemaran *Staphylococcus aureus* pada Susu Kambing Peranakan Etawa (PE) di Peternakan Adoe A Aceh Besar," *Jurnal Ilmiah Mahasiswa Veteriner (JIMVET)*, vol. 6, no. 3, pp. 89–95, 2022.

[43] J. G. Cappuccino, *Microbiology a laboratory manual*, 12th ed. San Francisco: Pearson Education Inc. Publishing as Benjamin Cummings, 2019.

[44] S. N. Aritonang, E. Roza, and E. Rossi, *Probiotik dan Prebiotik dari Kedelai untuk Pangan Fungsional*. Sidoarjo: Indomedia Pustaka, 2019.

[45] A. M. Kartikasari, I. S. Hamid, M. T. E. Purnama, R. Damayanti, F. Fikri, and R. N. Praja, "Isolasi dan Identifikasi Bakteri *Escherichia coli* Kontaminan pada Daging Ayam Broiler di Rumah Potong Ayam Kabupaten Lamongan," *Jurnal Medik Veteriner*, vol. 2, no. 1, pp. 66–71, Apr. 2019, doi: 10.20473/jmv.vol2.iss1.2019.66-71.

[46] S. Kursia, Imrawati, A. Halim, Sasmita, and F. Hanifah, "Identifikasi Biokimia dan Aktivitas Antibakteri Isolat Bakteri Asam Laktat dari Limbah Sayur Sawi (*Brassica juncea L.*)," *Jurnal Kesehatan*, vol. 1, no. 1, pp. 51–60, 2019.

[47] N. A. Lutfiah, "Isolasi dan Identifikasi Bakteri Asam Laktat pada Susu Kambing Saanen (*Capra aegagrus H.*)," Universitas Islam Negeri Maulana Malik Ibrahim, Malang, 2015.

[48] N. K. Srimurtini, N. Mastra, and J. Sofiyanti, "Identifikasi *Staphylococcus aureus* pada Rongga Mulut Mahasiswa dengan Karang Gigi di Jurusan Teknologi Laboratorium Medis Poltekkes Kemenkes Denpasar," Poltekkes Kemenkes Denpasar, Denpasar, 2020.

[49] G. S. Gandadimaja, "Keanekaragaman Bakteri Kamar Kos Mahasiswa di Daerah Babarsari dan Seturan Yogyakarta," Universitas Atma Jaya, Yogyakarta, 2023.

[50] Hafsan, *Prospek dan Perkembangan Bioteknologi Modern*. Depok: Rajawali Pers, 2022.

[51] B. Ramadhan and P. R. Wikandari, "*Review* Artikel: Aktivitas Enzim Amilase dari Bakteri Asam Laktat (Karakteristik dan Aplikasi)," *UNESA Journal of Chemistry*, vol. 10, no. 2, pp. 109–120, 2021.

[52] C. M. Putri, M. M. Rustama, and W. S. Putranto, "Skrining Bakteri Asam Laktat dan Khamir Potensial Proteolitik Ekstraseluler dan *Milk Clotting Activity* dari Ekstrak dan *Fresh Cheese* Stroberi (*Fragaria x ananassa Duch.*)," *Jurnal Teknologi Hasil Peternakan*, vol. 5, no. 1, pp. 61–82, Mar. 2024, doi: 10.24198/jthp.v5i1.49925.

[53] Y. S. Putri, "Skrining dan Uji Aktivitas Enzim Protease Bakteri dari Limbah Rumah Pemotongan Hewan," Universitas Airlangga, Surabaya, 2012.

[54] S. T. Cowan, *Cowan and Steel's Manual for the Identification of Medical Bacteria*. Cambridge: Cambridge University Press, 1993.

[55] B. Ardiyantoro, *Bakteriologi*. Cilacap: PT. Media Pustaka Indo, 2025.

[56] M. A. Gani, T. E. Tallei, and Fatimawali, "Identifikasi Bakteri Asam Laktat dari Hasil Fermentasi Selada Romain (*Lactuca sativa var.*

longifolia Lam.) Menggunakan Gen 16S rRNA," *PHARMACON*, vol. 8, no. 1, pp. 57–64, 2019.
[57] Y. Safitri and L. S. Gede, "Identifikasi Jenis Sampel (Bakteri Murni dan Campuran Bakteri) Penyebab ISK terhadap Hasil Uji *Sensitivity* Antibiotik *Ciprofloxacin*," *Jurnal Analis Medika Bio Sains*, vol. 4, no. 1, pp. 12–16, 2017.
[58] A. Ishak, N. Mazonakis, N. Spernovasilis, K. Akinosoglou, and C. Tsioutis, "Bactericidal versus bacteriostatic antibacterials: clinical significance, differences and synergistic potential in clinical practice," *J Antimicrob Chemother*, vol. 80, no. 1, pp. 1–17, Jan. 2025, doi: 10.1093/jac/dkae380.
[59] E. D. Pamungkas, "Uji Aktivitas Antibakteri Ekstrak Etanol Kulit Bawang Merah (*Allium ascalonicum*) terhadap Bakteri *Staphylococcus aureus*," STIKES Mitra Keluarga, Bekasi, 2021.
[60] S. R. Rahayuningsih, S. S. Patimah, T. Mayanti, and M. M. Rustama, "Aktivitas Antibakteri Ekstrak n-Heksana Daun Mangrove (*Rhizospora stylosa Griff*) terhadap Bakteri Patogen pada Ikan Nila (*Oreochromis niloticus*)," *J Mar Res*, vol. 12, no. 1, pp. 1–6, Jan. 2023, doi: 10.14710/jmr.v12i1.35657.
[61] A. Khan, W. R. Miller, D. Axell-House, J. M. Munita, and C. A. Arias, "Antimicrobial Susceptibility Testing for Enterococci," *J Clin Microbiol*, vol. 60, no. 9, pp. 1–20, Sep. 2022, doi: 10.1128/jcm.00843-21.
[62] S. Irmayanti, "Uji Efektivitas Antibakteri Ekstrak Etanol Daun Melinjo (*Gnetum gnemon L.*) terhadap Pertumbuhan Bakteri *Shigella dysenteriae* Penyebab Diare," Universitas Islam Negeri Maulana Malik Ibrahim Malang, Malang, 2024.
[63] H. Hanchi, W. Mottawea, K. Sebei, and R. Hammami, "The genus Enterococcus: Between probiotic potential and safety concerns-an update," *Front Microbiol*, vol. 9, no. 1, pp. 1–16, Aug. 2018, doi: 10.3389/fmicb.2018.01791.
[64] N. Agustiningsih, "Aktivitas Antibakteri dari Isolat Bakteri Probiotik yang Terdapat pada Saluran Pencernaan Ikan Lele (*Clarias Sp.*) terhadap Bakteri *Escherichia coli* dan *Staphylococcus aureus*," UIN Sunan Ampel Surabaya, Surabaya, 2023.
[65] M. R. Firdaus, A. E. Putra, and A. Abdiana, "Potensi Aktivitas Bakteriosin *Lactobacillus gasseri* terhadap Pertumbuhan *Salmonella typhi*," *Jurnal Ilmu Kesehatan Indonesia*, vol. 1, no. 3, pp. 314–320, May 2021, doi: 10.25077/jikesi.v1i3.80.
[66] R. T. Manalu, S. Bahri, and S. Sarah, "Isolasi dan Karakterisasi Bakteri Asam Laktat asal Feses Manusia sebagai Antibakteri *Escherichia coli* dan *Staphylococcus aureus*," *Sainstech Farma*, vol. 13, no. 1, pp. 55–59, 2020.
[67] M. S. Artha, "Pertumbuhan Bakteri Asam Laktat *Enterococcus faecium* Sebagai Penghasil Bakteriosin pada Kondisi Aerob dan Anaerob," Universitas Mataram, Mataram, 2016.