ISSN: 18158129 E-ISSN: 18151027
Volume 18, Issue 07, July, 2022

Microbiological quality analysis and molecular identification of smoked skipjack tuna in market west Papua

Sipriyadi[1], Sukmawati Sukmawati[2], M Iksan Badaruddin[3], Melda Yunita[4], Akhmad Ali[5], Erni Angraini[6]

Department of Biology, Faculty of Mathematics and Natural Science, Universitas Bengkulu, Bengkulu, Sumatera, Indonesia[1]
Master's Study Program in Biology, Faculty of Mathematics and Natural Science, Universitas Bengkulu, Bengkulu, Sumatera, Indonesia[1]
Department of Fishery Product Processing, Faculty of Fishery, Universitas Muhammadiyah Sorong, Sorong, West Papua, Indonesia[2,3]
Department of Medical Education, Faculty of Medicine, Universits Pattimura. Jl. Ir. M. Putuhena Ambon, Maluku, Indonesia[4]
Department of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Sorong, West Papua, Indonesia[5]
Department of Biology Education, Faculty of Teacher Training and Education, Universitas Muhammadiyah Palembang, Palembang, South Sumatera, Indonesia[6]

ABSTRACT— The production of fishery products in the city of Sorong, was recorded at 44,710 tons in 2017, one of which was smoked skipjack tuna. The smoking of fish is done through the process of salting, and providing smoke. The purpose of smoking is to prevent damage to fish due to the process of decreasing water content. The unique characteristics of smoked fish are caused by the presence of volatile compounds that are easily vaporized and trapped in the fish flesh. The smoking process in fish affects the physical, chemical, microbiological and organoleptic properties of the product. The objective of this study was to determine the microbiological quality of smoked fish and to identify bacteria molecularly in smoked skipjack tuna in the market. The method used in this study is a descriptive method to describe the total plate count of microbes, examination *of E. coli* and *Salmonella,* as well as molecular identification of bacteria found in smoked skipjack tuna in market. The results showed that all samples indicated the presence of microbial TPC, examination results of *E. coli* and *Salmonella* were declared not to exceed the maximum limit according to Indonesian national standards. As for molecular identification, it was found that sample Ca.1 was identified as *Bacillus thuringiensis* BD17-E12, sample Ca.2 was identified as *Bacillus cereus* MD152, and sample Ca.3 was identified as *Bacillus thuringiensis* ucsc27.

KEYWORDS: Microbiological Quality, Bacteria Identification, Smoked Skipjack.

1. INTRODUCTION

Fish is an animal food that is widely consumed by the people of Indonesia because fish has a high nutritional content such as protein, fat, minerals and vitamins. These nutrients are needed by humans. The production of fishery products in the city of Sorong, West Papua, was recorded at 44,710 tons in 2017, one of which is smoked skipjack tuna [1]. The smoking of fish is carried out through the process of salting, drying, and providing sap. The purpose of smoking is to prevent fish damage due to the process of decreasing the water content of the product [2]. Some of the advantages of smoked fish products made traditionally include

producing attractive colors, as well as distinctive textures and flavors [3].

The unique characteristics of smoked fish are caused by the presence of volatile compounds that easily evaporate and are trapped in the fish flesh [4- 6]. The smoking process in fish affects the physical, chemical, microbiological and organoleptic properties of the product. The physical, chemical, organoleptic, and microbiological properties of smoked fish products are influenced by raw materials, such as the use of fresh or frozen fish [7].

The principle of smoking for food preservation is to reduce the water content of a product. High water content in a product can spoil in a short time. This is caused by spoilage microorganisms such as bacteria and fungi. Through the smoking process, there will be a decrease in the water content so that microorganisms cannot grow [8]. During the smoking process, an oxygen-free environment is also formed so that aerobic bacteria cannot grow [9], [10].

In addition to reducing the water content of smoked fish products, the smoking process also gives unique characteristics to fish, including a distinctive aroma and taste. The smoke produced from firewood during combustion is a group of compounds such as water, aldehydes, acetic acid, ketones, alcohol, formic acid, phenol, and CO_2. These compounds enter and are trapped in the fish meat so as to create the distinctive aroma and taste of smoked fish [11], [12].

A study on smoked fish including smoked tuna loin produced in Air Manis Hamlet, Laha Village was declared to meet the microbiological quality requirements according to standart national Indonesia [13], with a total microbe of $8.5x10^1$ CFU/g and no pathogenic microbes such as *E. coli* or *Salmonella* were detected, and the water content of smoked tuna loin was 59% and with a pH of 5.8 [14].

Another study reported that smoked skipjack tuna sold in the traditional market had the Total Plate Count (TPC) of the three samples observed, sample one was 135×10^6 CFU/g, sample 2 was 13×10^6 CFU/g, while sample 3 is 275×10^6 CFU/g. In addition, there was also *Vibrio cholera* in sample 1 which was 140×10^1, sample 2 was 90×10^1 CFU/g, and sample 3 was 190×10^1 CFU/g while the examination result of *Staphylococcus aureus* in sample 1 was 6×10^6 CFU/g, sample 2 could not be calculated and sample 3 was 56×10^6 CFU/g [15]. The results of this study have passed the Indonesian National Standard [13] while the results of the examination of *Salmonella typhi* and *Escherichia coli* did not show colony growth on the media [15]. Furthermore, according [16] in their research, smoked tuna did not find *Salmonella*, yet found *Staphylococcus aereus* with an average colony number of $3.5x10^4$ CFU/g. Based on the background description, it is necessary to identify bacteria at the molecular level in smoked skipjack tuna products. Previous studies have never identified pathogenic bacteria in smoked fish products at the molecular level, but only limited to growing bacteria on selective media. The objective of this study was to determine the microbiological quality and to identify bacteria molecularly in smoked skipjack tuna in the market, West Papua.

2. Material and Methods

2.1 Research design

The study was carried out from April to December 2021 at the Laboratory of the Faculty of Fisheries, Muhammadiyah University of Sorong and the Biology Laboratory of Bengkulu University. Samples of smoked skipjack tuna were taken from the market, West Papua. Sampling was done randomly at three different points, and was repeated three times.

The method used is a descriptive method, which includes a total microbial plate count test using the pour method using Nutrient Agar (NA) media, the coliform test using the three-tube series technique as an presumptive test using Lactose broth (LB) media, while the confirmed test using the medium eosin methylene blue agar (EMBA) and Salmonella test using Salmonella shigella agar (SSA) media. Furthermore, molecular identification was carried out using the PCR-colony technique.

2.2 Isolation of Genomic DNA of Bacterial Isolates [17]

A total of 1.5 mL of bacterial culture was centrifuged at 8000 rpm for 10 minutes, then the pellet was washed with STE buffer (0.3 M sucrose; 25 mM Tris-HCL; 25 mM EDTA.2Na pH 8), then centrifuged at 8000 rpm for 10 minutes. Pellets were washed 3 times repeatedly. The pellets were added with 200 L of STE buffer and 45 L of lysozyme (20 mg/mL) and then incubated at 55 ºC for 1 hour to form protoplasts.

A total of 20 L proteinase-K (20 mg/mL) was added to the mixture and incubated at 55 ºC for 60 minutes. Then 400 L 10% CTAB was added in a solution of 0.7 M NaCl then incubated at 65 ºC for 30 minutes and put into a phenol:chloroform solution (25:24), then centrifuged at 12000 rpm for 10 minutes. The clear phase was put into a new tube and added 0.6 times the volume of isopropanol and 20 L of sodium acetate, then incubated at -20 ºC for one night. Then it was centrifuged at 12000 rpm for 10 minutes. The pellets were washed using 1 mL of alcohol 70%. The DNA was dried for 1 hour and then dissolved in 50 L sterile ddH2O, then the results of DNA isolation was stored at 4 C or -20ºC.

16S rRNA Gene Amplification of Bacterial Isolates

The 16S rRNA gene from genomic DNA was amplified by a Polymerase Chain Reaction (PCR) machine using a prokaryotic-specific primer [18], namely the forward primer 63f (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and reverse primer 1387r. (5′-GGG CGG WGT GTA CAA GGC-3′). The PCR conditions used were pre-denaturation, denaturation, annealing, elongation and post-PCR with a total of 30 cycles. Separation of PCR product DNA using 1% agarose at 75 volts for 45 minutes. DNA visualization was performed on a UV transluminator.

Data Sequencing and Analysis

Data resulted from the sequencing were then trimmed and assembled using the ChromasPro version 1.5 program. The data were performed with BLAST to genomic data that have been registered by NCBI/ National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). The data were reanalyzed by aligning the sequences using the MEGA 5.0 program and a phlogenetic tree construction was carried out to show the degree of relatedness between isolate Xyl_22 with actinomycetes and other non-catinomycetic microbes using the Naighbor Joining Tree method with 1000 replicates boostrap.

3. Results

The results of the microbial total plate count (TPC) test and the examination of E. coli on 3 samples of smoked skipjack tuna that were examined with 3 times of data collection showed that the average number of total microbial plate counts did not exceed the maximum standard limit according to the Indonesian National Standard [13], as well as for the results of the *E. coli* examination (Table 1). Meanwhile, the morphological characteristics, from color to shape, show similar characteristics.

Table 1. The results of the total plate count (TPC), examination of *E. coli*, examination of *Salmonella* on smoked skipjack tuna products at the Remu market, Sorong City

First Week			
Sampel	TPC (CFU/g)	*E. coli* Examination	*Salmonella* Examination

		Presumptive Test (MPN/g)	Conformed Test (MPN/g)	
Ca.1.1	2.88 x 10^3	3	0	0
Ca.1.2	7.20 x 10^3	3	0	0
Ca.1.3	1.84 x 10^4	3	0	0
Second Week				
Ca.2.1	2.0 x 10^2	6.1	0	0
Ca.2.2	4.5 x 10^3	6.1	0	0
Ca.2.3	2.4 x 10^4	6.1	0	0
Third Week				
Ca.3.1	2.3 x 10^2	3	0	0
Ca.3.2	1.5x 10^3	3	0	0
Ca.3.3	3.6 x 10^4	3	0	0

Note: The Indonesian National Standards for smoked fish products are: maximum TPC 5x10^5 CFU/g, maximum *E. coli* is < 3 MPN/g, and Salmonella is negative or 0.

Genome amplification of smoked skipjack tuna samples by PCR method using primers 63f and 1387r resulted in DNA fragments measuring about 1300 bp (Figure 1).

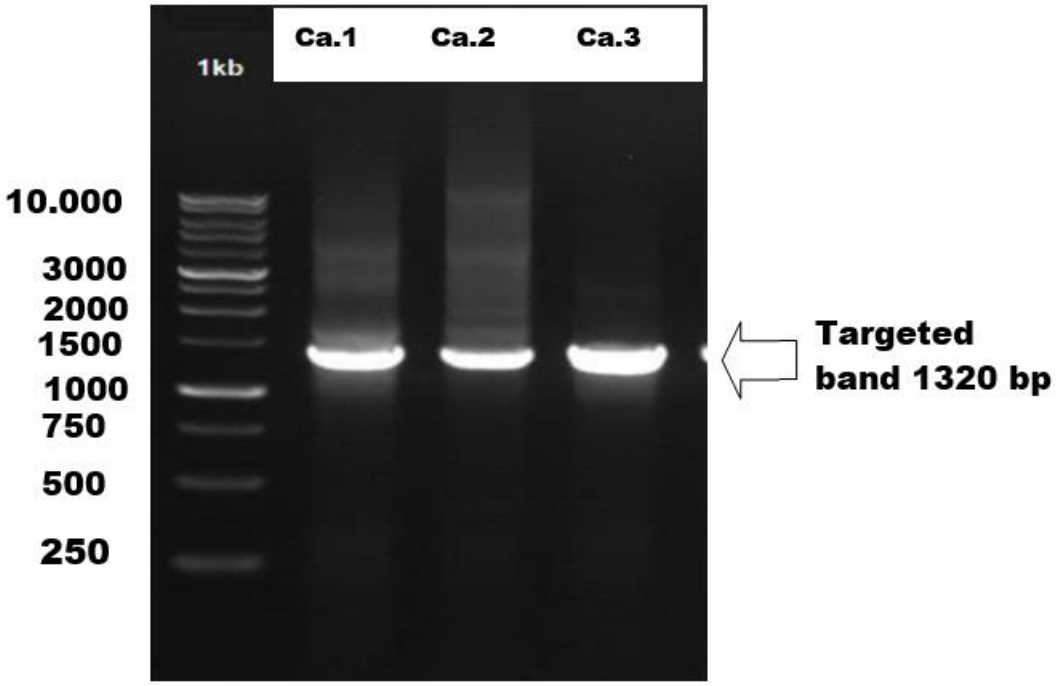

Figure 1. PCR amplification of the 16S rRNA gene using primers 63f and primers 1387r; M = marker 1 Kb ladder; lane 1-3 = PCR product of isolate Ca.1, Ca.2, and Ca.3

3.1 The sequence of the 16S rRNA gene of Isolate Ca.1

TGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATAC
CGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGAT
GGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCG
ACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAG
GCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACC

TTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
TGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGT
GAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGG
AAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAA
GGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGA
TACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTG
CTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGA
ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCT
TACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGA
CAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGTGACAACCG
GAGGAAGGGGGGATGACGTCAATCATCATGCCCCTTATGAC

Table 2. The results of the alignment of the 16S rRNA gene sequences of isolate Ca.1 to the data available at NCBI (BLASTX)

Isolate Code	Accession Number	Description	Identity	E value
Isolate Ca.1	HF584771.1	***Bacillus thuringiensis* partial 16S rRNA gene, isolate BD17-E12**	99.73 %	0.00
	MK648334.1	*Bacillus cereus* strain 1.4PT6 16S ribosomal RNA gene, partial sequence	99.73 %	0.00
	MT605498.1	*Bacillus pacificus* strain WS1-1 16S ribosomal RNA gene, partial sequence	99.64 %	0.00
	MT372153.1	*Bacillus manliponensis* strain YEBN5 16S ribosomal RNA gene, partial sequence	98.64 %	0.00

3.2 The sequence of the 16S rRNA gene of Isolate Ca.2

GGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTT
CGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGTGAGGTAACGGCTCACCAAG
GCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAG
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG
CCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAG
TTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAG
CCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTG
GTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGA
CTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGA
GGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGC
GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAG
GGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCA
AGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCG
AAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCT
CCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGG
TTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGG
TGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGAC
CTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTA
ATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCG
CTAGT

Table 3. The results of the alignment of the 16S rRNA gene sequences of isolate Ca.2 to the data available at NCBI (BLASTX)

Isolate Code	Accession Number	Description	Identity	E value
Kultur Isolat Ca.2	MT642947.1	***Bacillus cereus* strain MD152 16S ribosomal RNA gene, partial sequence**	99.83 %	0.00
	MT611943.1	*Bacillus tropicus* strain ISP161A 16S ribosomal RNA gene, partial sequence	99.83 %	0.00
	MW741611.1	*Bacillus albus* strain ASSF01 16S ribosomal RNA gene, partial sequence	99.83 %	0.00
	MW405818.1	*Bacillus paranthracis* strain YBT2 16S ribosomal RNA gene, partial sequence.	99.83 %	0.00

3.3 The sequence of the 16S rRNA gene of Isolate Ca.3

AAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTG
AGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGG
GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGA
AAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTA
GGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACG
GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGG
CGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGG
GTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTG
AAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACAC
TGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG
ATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCG
CCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAA
ACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGC
TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCA
TTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAACCGGAGGAAGGTGGGATGACGTCAATC
ATCATGCCCCTTATGACTGGGCTACCACGTGCTACATGGACGTACAAGAACTGCAGAACGCCA
GGTGGA

Table 4. The results of the alignment of the 16S rRNA gene sequences of isolate Ca.3 to the data available at NCBI (BLASTX)

Isolate Code	Accession Number	Description	Identity	E value
Isolate Ca.3	FN667913.1	***Bacillus thuringiensis* partial 16S rRNA gene, strain ucsc27**	99.26 %	0.00
	MT256061.1	*Bacillus wiedmannii* strain B16-2 16S ribosomal RNA gene, partial sequence	99.17%	0.00
	MK648334.1	*Bacillus cereus* strain 1.4PT6 16S ribosomal RNA gene, partial sequence	99.25 %	0.00
	MW642047.1	*Bacillus tropicus* strain WHS116 16S ribosomal RNA gene, partial sequence	99.16 %	0.00

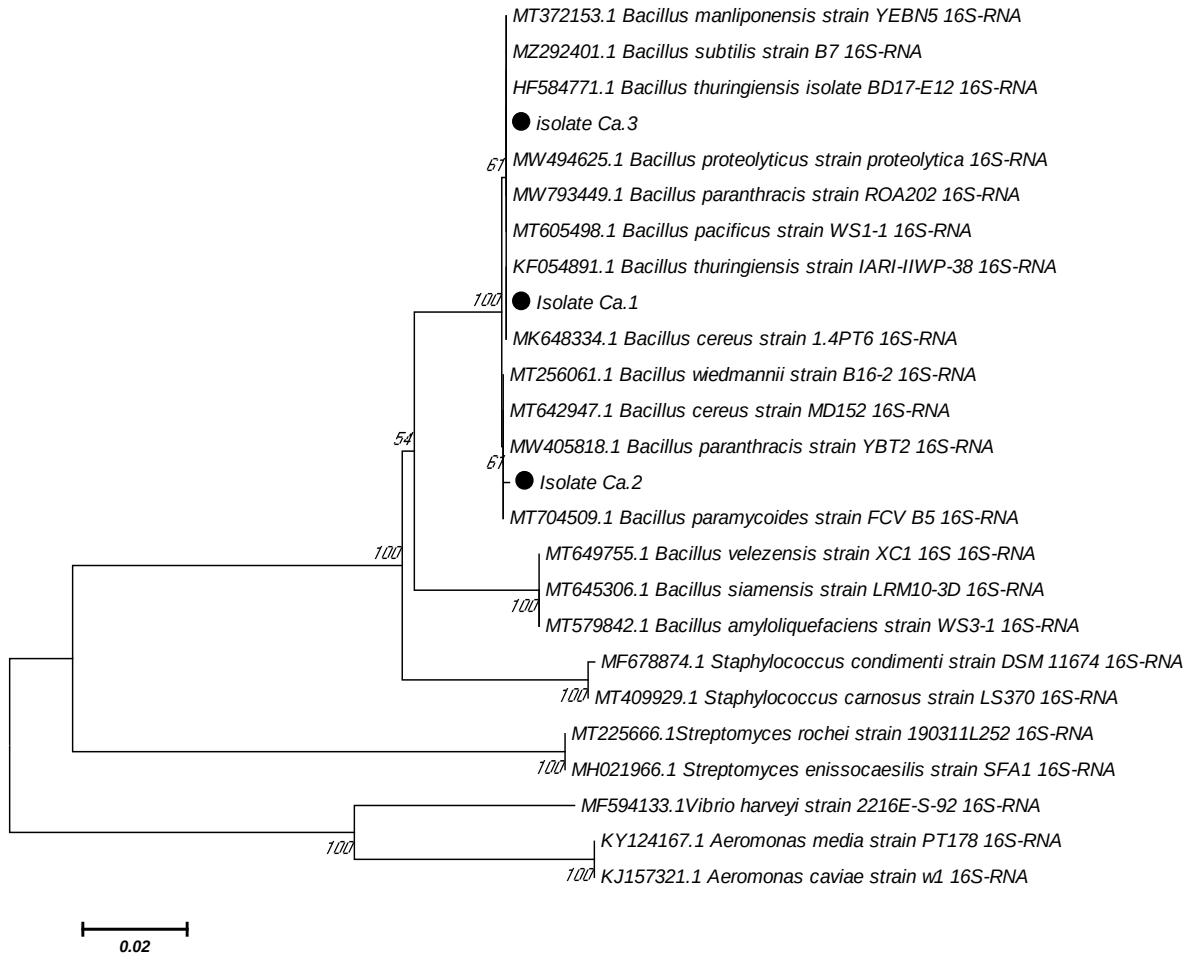

Figure 2. Phylogenetic tree depicting the relatedness of isolate Ca.1, Ca.2, and Ca.3 to other bacteria in one clade and to other clades (outer group). The construction is based on the Neighbor Joining Tree method with a bootstap value of 1000x replications.

4. Discussion

Based on the results of the study, it showed that the total microbial plate count in the first week for sample Ca.1.1, sample Ca.1.2, and sample ca.1.3 had a total microbial plate count (TPC) of $2.88x10^3$ CFU/g, $7.2x10^3$ CFU/g, and $1.8x10^4$ CFU/g, respectively. Meanwhile, the *E. coli* and *Salmonella* examinations for the three samples from the first week to the third week were 0 or negative (Table 1).

Then in the second week, the total microbial plate count (TPC) in the Ca.2.1 sample was $2.0x10^2$ CFU/g, the Ca.2.2 sample was $4.5x10^3$ CFU/g, and the Ca.2.3 sample was $2.3x10^2$ CFU/g (Table 1). The results of observations in the third week for sample Ca.3.1 was $2.3x10^2$ CFU/g, sample Ca.3.2 was $1.5x10^3$ CFU/g, and sample Ca.3.3 was $3.6x10^4$ CFU/g. while the *E. coli* examination for all these samples was 0 MPN/g.

Meanwhile, all samples from the first week to the third week showed similar morphological characteristics, including white colony color, convex elevation, smooth margin, and rod shape (Table 1).

According to the observation results of the microbial total plate count (TPC) and the examination of *E. coli* and *Salmonella*, it can be stated that all samples observed did not exceed the maximum standard limit [13]. Contamination of pathogenic microbes in processed fish products can be caused by several factors, such as

raw materials are not in fresh condition, the total number of microbes in the initial conditions has exceeded the maximum limit, unhygienic processing processes such as washing water contaminated by pathogenic bacteria, non-standardized packaging, unhygienic product sales place, product distribution is not in accordance with operational standards, product storage duration, and environmental factors including high humidity and temperature. While the identification of bacteria found in smoked skipjack tuna to the genetic level was carried out on one sample as a representative for each week. This was done because the colony characteristics for each sample were similar.

As for molecular identification, PCR amplification for sample Ca.1, sample Ca.2 and sample Ca.3 showed a band size of 1,320 bp. This is in accordance with the primary size used (Figure 1). Furthermore, the results of sequencing and construction of phylogenetic trees showed that sample Ca.1 was identified as *Bacillus thuringiensis* partial 16S rRNA gene isolate BD17-E12 (Table 2; Figure 2), sample Ca.2 was identified as *Bacillus cereus* strain MD152 16S ribosomal RNA gene partial sequence (Table 3; Figure 2), and sample Ca.3 was identified as *Bacillus thuringiensis* partial 16S rRNA gene strain ucsc27 (Table 4; Figure 2).

Bacillus thuringiensis is a Gram-positive, rod-shaped, facultative aerobic species of bacteria and is widely distributed in various countries [19]. Several studies have shown that *Bacillus thuringiensis* is one of the safe and recommended biological controllers for mosquito control. *B. thuringiensis* can form spores and has the ability to form protein crystals that are toxic to insects [20], [21]. *B. thuringiensis* has the potential as a biopesticide and bioinsecticide in eradicating mosquito larvae [22], [23]. *B. thuringiensis* can also be used as a biodegradation material for colored plastic waste [24]. These bacteria have a wider environmental coverage because of their facultative aerobic nature [25]. Therefore, it is possible that *B. thuringiensis* is also present in smoked skipjack tuna products. In addition, *B. Thuringiensis* has the ability to fix non-symbiotic nitrogen around the roots of rice plants [26] so that these bacteria have the potential as biofertilizers. *B. thuringiensis* can also be used as a biocontrol agent against tobacco plants [27]. However, stated that *B. thuringiensis* is a food-borne disease [28].

B. thuringiensis is also found in lettuce, kimbap, and spinach sold in South Korea [29]. *Bacillus thuringiensis* is commonly detected in soil. These bacteria have very similar genotypes and phenotypes. *B. thuringiensis* and *B. cereus* are also frequently found in food. The presence of *B. thuringiensis* is often found in food products because it is known that *B. thuringiensis* is used as a biopesticide in the cultivation of certain plants. In addition to biopesticides, they are also often used as bioinsecticides [30]. Several previous studies have also reported that *B. thuringiensis* has been detected in foods such as milk, fresh fruits and seeds [31].

Furthermore, *Bacillus cereus* is a rod-shaped bacterium, and is commonly found in some food retailers in Nigeria [32]. *B. cereus* is a bacterial pathogen that causes foodborne desease. Foodborne diseases caused by *B. cereus* are generally triggered by emetic toxins, where these toxins can cause vomiting in sufferers. In addition, there are also enterotoxins that cause diarrhea. Furthermore, there is also cytotoxin K (CytK). The expression of B. cereus toxins Hb1, Nhe, and CytK is regulated by the quorum-sensing PlcR system. *B. Cereus* is able to produce spores under extreme conditions. *B. cereus* spores are an important factor contributing to foodborne desease. Spores of *B. cereus* are more hydrophobic than spores of *Bacillus* spp. thus the ability to stick to several types of surface is greater. However, foodborne desease caused by *B. cereus* has relatively mild symptoms with a shorter duration [33], [34].

Vomiting and diarrhea caused by *B. cereus* releases cereulide peptides and protein enterotoxins such as hemolysin BL, nonhemolytic enterotoxin, and K cytotoxin. Furthermore, *B. cereus* is also a foodborne pathogen and causative agent of non-gastrointestinal infections and nosocomial complications [35].

According to the report of Agata [36] in their research, the production of emetic toxin (cereulide) caused by *B. cereus* in food samples indicated by food poisoning, may cause vomiting ranging from 0.01 to 1.28 g/g.

In addition, the emetic type strain *B. cereus* NC7401 was inoculated into various food products and then incubated for 24 hours at 30 °C and 35 °C. The results showed that *B. cereus* rapidly increased to 10^7–10^8 CFU/g and produced an emetic toxin. Furthermore, low levels of emetic toxins according to the results of their research are found in eggs, meat, liquid foods including milk and soy milk. Meanwhile, bacterial growth and toxin production are inhibited in foods that are processed using mayonnaise, vinegar, and sauces. This can occur due to a decrease in pH by acetic acid [36]. *B. cereus* is the second risk factor for foodborne diseases in fresh agricultural products, while bacterial contamination of *B. cereus* is one of the main problems in vegetables [37].

Bacterial contamination in smoked fish is influenced by environmental factors, conditions after smoked fish are processed, stored carelessly in baskets both during processing and selling, and the absence of air exchange, thus causing humid conditions. In humid conditions, pathogenic bacteria have a very high growth ability [38], [39]. Bacterial growth can also occur when the fish is processed, during the storage process and distribution to consumers, besides the seller also does not pay attention to the sanitation and hygiene of the product [40].

Preserving fish by smoking can reduce bacterial growth [41]. However, the possibility of contamination with pathogenic bacteria can still occur during and after the processing. The presence of pathogenic bacteria in fish or the metabolic products of these bacteria can cause health problems in the form of poisoning in the form of intoxication and infection [42]. The main causes of contamination include processing procedures, distribution, and sales in unhygienic markets [43]. In addition, the product packaging process also affects the bacteria found in smoked fish [44]. It is known that human hands are a source of bacterial contamination from wounds or skin infections so that processed foods are very closely related to humans who handle them [45].

The microbiological quality of smoked skipjack tuna at the market, West Papua shows that the total plate count (TPC) of microbes, *E. coli and Salmonella* stated for all samples observed did not exceed the maximum limit and was in accordance with the Indonesian National Standard. While molecular identification showed that sample Ca.1 was identified as *Bacillus thuringiensis* partial 16S rRNA gene isolate BD17-E12, sample Ca.2 was identified as *Bacillus cereus* strain MD152 16S ribosomal RNA gene partial sequence, and sample Ca.3 was identified as *Bacillus thuringiensis* partial 16S. rRNA gene strain ucsc27.

5. References

[1] BPS. (2021). Badan Pusat Statistik Kota Sorong. https://sorongkota.bps.go.id/indicator/56/270/1/produksi.html.

[2] Michael, S.E., Cai, J., Akwasi, A.Y. and Adele, A. (2019). Fish Smoking in Ghana: a review. J. of fisheries sciences. Vol. 13(3). pp: 0-0.

[3] Berhimpon, S., Montolalu, R.I., Dien, H.A., Mentang, F. and Meko, A.U.I. (2018). Concentration and Application Methods of Liquid Smoke for Exotic Smoked Skipjack (Katsuwonus pelamis L.). I. Food Research J. Vol. 25(5).

[4] Varlet, V., Prost, C. and Serot, T. (2007). Volatile Aldehydes in Smoked Fish: Analysis Methods, Occurence and Mechanisms of Formation. Food chemistry. Vol. 105(4). pp:1536-1556.

[5] Jónsdóttir, R., Ólafsdóttir, G., Chanie, E. and Haugen, J.E. (2008). Volatile compounds suitable for rapid detection as quality indicators of cold smoked salmon (Salmo salar). Food chemistry. Vol. 109(1). pp:184-195.

[6] Feng, M., Dai, Z., Yin, Z., Wang, X., Chen, S. and Zhang, H. (2021). The Volatile Flavor Compounds of Shanghai Smoked Fish as a Special Delicacy. J. of food biochemistry. Vol.45(1). pp:e13553.

[7] Arvanitoyannis, I.S. and Kotsanopoulos, K.V. (2012). Smoking of Fish and Seafood: History, Methods and Effects on Physical, Nutritional and Microbiological Properties. Food and Bioprocess Technology. Vol. 5(3). pp:831-853.

[8] Ibrahim, B.U., Baba, J. and Sheshi, M.S. (2014). Isolation and Identification of Bacteria Associated with Fresh and Smoked Fish (Clarias gariepinus) in Minna Metropolis, Niger State. Nigeria. J. of Applied and Environment Microbiology. Vol. 2(3). pp:81-85.

[9] Badr, H.M. (2012). Control of The Potential Health Hazards of Smoked Fish by Gamma Irradiation. I. J. of food microbiol. Vol. 154(3). pp:177-186.

[10] Al-Reza, S., Karmaker, S., Hasan, M., Roy, S., Hoque, R. and Rahman, N. (2015). Effect of Traditional Fish Processing Methods on The Proximate and Microbiological Characteristics of Laubuka Dadiburjori During Storage at Room Temperature. J. of fisheries and Aquatic Science. Vol. 10(4). pp: 232.

[11] Daramola, J.A., Fasakin, E.A. and Adeparusi, E.O. (2007). Changes in Physicochemical and Sensory Characteristics of Smoke-dried Fish Species Stored at Ambient Temperature. African journal of food, agriculture, nutrition and development. Vol. 7(6).

[12] Sayuti, M., Salampessy, R.B.S., Asriani, A., Nurbani, S.Z. and Saidin, S. (2022). Chemical and Hedonic Characteristics of Smoked Katsuwonus pelamis (Fufu Fish) from Sorong, West Papua, Indonesia. Biodiversitas J. of Biological Diversity. Vol. 23(3).

[13] BSNI. (2009). Batas Maksimum Cemaran Mikroba dalam Pangan BSNI 7388:2009.

[14] Mailoa, M.N., Lokollo, E., Nendissa, D.M. dan Harsono, P.I. (2019). Microbiological and Chemical Characteristics of Smoked Tuna. JPHPI. Vol. 22(1). pp: 89-99.

[15] Yusmita, S. and Syarif, S. (2019). Identifikasi Bakteri Patogen Pada Ikan Cakalang (Katsuwonus pelamis) Asap Di Pasar Tradisional Kota Kendari. J. medilab mandala waluya kendari. Vol. 3(2).

[16] Majid, A., Majid, N. (2021). Isolation and identification of Salmonella and Staphylococus aureus Bacteria in Smoked Tuna Stored at Different Temperatures and Storage Periods. CHMK Applied Scientific J. Vol. 4(2). pp:63-72.

[17] Sambrook, J. and Russell, D.W. (2001). Molecular Cloning a Laboratory Manual. 3rd ed. New York. Cold Spring Harbor Laboratory Pr.

[18] Marchesi, J.R. 1998. Design and Evaluation of Useful Bacterium-Spesific PCR Primers that Amplify Genes Coding for Bacterial 16S rRNA. Appl environ microbial. 64. pp:795-799.

[19] Marchlewicz, A., Domaradzka, D., Guzik, U., Wojcieszyńska, D. (2016). Bacillus thuringiensis B1 (2015b) is a Gram-Positive Bacteria Able to Degrade Naproxen and Ibuprofen. Water, Air, & Soil Pollution. Vol. 227(6). pp: 1-8.

[20] Aldeewan, A., Zhang, Y. and Su, L. (2014). Bacillus thuringiensis Parasporins Functions on Cancer Cells. I. J. Pure Appl Biosci. Vol. 2. pp:67-74.

[21] Adang, M.J. (2018). Bacillus thuringiensis Insecticidal Crystal Proteins: Gene Structure, Action, and Utilization. In Biotechnology for Biological Control of Pests and Vectors, CRC Press: 3-24.

[22] Kovendan, K., Murugan, K., Vincent, S. and Barnard, D.R. (2012). Studies on Larvicidal and Pupicidal Activity of Leucas Aspera Willd. (Lamiaceae) and Bacterial Insecticide, Bacillus sphaericus, Against Malarial Vector, Anopheles Stephensi Liston. (Diptera: Culicidae). Parasitology Research. Vol. 110(1). pp:195-203.

[23] Bordalo, M.D., Gravato, C., Beleza, S., Campos, D., Lopes, I. and Pestana, J.L.T. (2020). Lethal and Sublethal Toxicity Assessment of Bacillus thuringiensis var. Israelensis and Beauveria bassiana Based Bioinsecticides to The Aquatic Insect Chironomus Riparius. Science of the Total environment 698:134155.

[24] Meena, R.P., Ghosh, S., Jatav, S.S., Chitara, M.K., Jinger, D., Gautam, K. and Parihar, M. (2021). Microbial Mediated Biodegradation of Plastic Waste: an Overview. Bioremediation Science from Theory to Practice. pp: 154-169.

[25] Hou, L. and Majumder, E.L.W. (2021). Potential For and Distribution of Enzymatic Biodegradation of Polystyrene by Environmental Microorganisms. Materials. Vol. 14(3). pp:503.

[26] Gouda, S., Kerry, R.G., Das, G., Paramithiotis, S., Shin, H.S, dan Patra, J.K. (2018). Revitalization of Plant Growth Promoting Rhizobacteria for Sustainable Development in Agriculture. Microbiological Research 206. pp:131-140.

[27] Blanc, M., Kaelin, P. and Gadani, F. (2002). Bacillus thuringiensis (BT) for The Control of Insect Pests in Stored Tobacco: A Review. Beiträge Zur Tabakforschung International/contributions to Tobacco Research. Vol. 20(1). pp:15-22.

[28] Bonis, M., Felten, A., Pairaud, S., Dijoux, A., Maladen, V., Mallet, L. and Herbin, S. (2021). Comparative Phenotypic, Genotypic and Genomic Analyses of Bacillus thuringiensis Associated with Foodborne Outbreaks in France. PloS one 16(2):e0246885.

[29] Wei, S., Chelliah, R., Park, B. J., Kim, S. H., Forghani, F., Cho, M. S. and Oh, D. H. (2019). Differentiation of Bacillus thuringiensis from Bacillus cereus group using a unique marker based on real-time PCR. Frontiers in microbiology. Vol. 10:883.

[30] Azmi, N.U., Ghafar, N.S.A., Yin, C.J., Yakubu, S., Adli, A.A. and Aziz, N.A.A. (2015). Toxicity of Bacillus thuringiensis Biopseticide Produced in Shrimp Pond Sludge as Alternative Culture Medium Against Bactocera dorsalis (Hendel). Acta biology Malaysia. Vol. 4. pp:5-16. Doi:10.7593/abm/4.1.5.

[31] Chiu, Y.H., Gaskins, A.J., Williams, P.L., Mendiola, J., Jørgensen, N. and Levine, H. (2016). Intake of Fruits and Vegetables with Low-to-moderate Pesticide Residues is Positively Associated with Semen-Quality

Parameters Among Young Healthy Men. Journal nutrition (146). pp:1084–1092. doi: 10.3945/jn.115.226563

[32] Adesetan, T.O., Efuntoye, M.O. and Babalola, O.O. (2020). Genotypic Profiling of Bacillus cereus Recovered From Some Retail Foods in Ogun State, Nigeria, and Their Phylogenetic Relationship. International Jour. of Microbial. //doi.org/10.1155/2020/3750948.

[33] Granum, P.E. and Lindbäck, T. (2012). Bacillus cereus, Food Microbiol: Fundamentals and Frontiers. pp:491-502.

[34] Ehling-Schulz, M., Knutsson, R. and Scherer, S. (2010). Bacillus cereus. Genomes of Foodborne and Waterborne Pathogens. pp: 147-164.

[35] Jovanovic, J., Ornelis, V.F., Madder, A. and Rajkovic, A. (2021). Bacillus cereus Food Intoxication and Toxicoinfection. Comprehensive Reviews in Food Science and Food Safety. Vol. 20(4). pp:3719-3761.

[36] Agata, N., Ohta, M. and Yokoyama, K. (2002). Production of Bacillus cereus Emetic Toxin (cereulide) in Various Foods. I. J of Food Microbial. Vol 73(1). pp: 23-27.

[37] Felício, M.D.S., Hald, T., Liebana, E., Allende, A., Hugas, M. and Nguyen-The, C. (2015). Risk Ranking of Pathogens in Ready-to-eat Unprocessed Foods of Non-animal Origin (FoNAO) in The EU: Initial Evaluation Using Outbreak Data (2007–2011). I. J food microbiol. (195). pp:9–19. doi: 10.1016/j.ijfoodmicro.2014.11.005.

[38] Hardianti, F. and Aziz, I.R. (2019). Identification of Pathogenic Bacteria on The Salted Fish Lutjanus vivanus in Sorong City of West Papua. Malaysian J. of microbiology. Vol. 15(3). pp: 237-244.

[39] Mendez, M., Rodríguez, R., Ruiz, J., Morales-Adame, D., Castillo, F., Hernández-Castillo, F.D. and Aguilar, C.N. (2012). Antibacterial Activity of Plant Extracts Obtained with Alternative Organics Solvents Against Food-Borne Pathogen Bacteria. Industrial Crops and Products. Vol.37(1). pp:445-450.

[40] Sukmawati, S., Badaruddin, I. and Simohon, E.S. (2020). Analysis of Total Plate Count Microba in Fresh (Rastrelliger sp.) Mackerel Fishof Sorong city West Papua. Samakia: Jurnal Ilmu Perikanan. Vol. 11(1). pp:10-14.

[41] Sampels, S. (2015). The Effects of Storage and Preservation Technologies on The Quality of Fish Products: A review. J. of Food Processing and Preservation. Vol. 39(6). pp:1206-1215.

[42] Ramos, S., Silva, V., Dapkevicius, M.D.L.E., Caniça, M., Tejedor-Junco, M.T., Igrejas, G. and Poeta, P. (2020). Escherichia coli as Commensal and Pathogenic Bacteria Among Food-Producing Animals: Health Implications of Extended Spectrum β-lactamase (ESBL) Production. Animals. Vol. 10(12). pp:22-39.

[43] Sukmawati, S., Badaruddin, I., Dewi, N.K., Situmorang, N., Mahfut, M. and Mustapa, F. (2021). Analysis of Organoleptic and Coliform Value in Fresh Mackerel (Rastrelliger Sp.) Fish in TPI Sorong City. In J. of Physics: Conference Series. Vol. 1764(1). pp:012035). IOP Publishing.

[44] Oğuzhan, Y.P. (2015). Effect of Essential Oils and Packaging on Hot Smoked Rainbow Trout During Storage. J. of Food Processing and Preservation. Vol. 39(6). pp:806-815.

[45] Afé, OHI., Saegerman, C., Kpoclou, Y.E., Douny, C., Igout A., Mahillon, J. and Scippo, M.L. (2021). Contamination of Smoked Fish and Smoked-dried Fish with Polycyclic Aromatic Hydrocarbons and Biogenic Amines and Risk Assessment for The Beninese Consumers. Food control. Vol. 126. pp 108089.