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Antifungal activities of *Lactobacillus brevis* strain NCTC13768 isolated from "budu fish" against spoilage fungi in dried fish

[1,*]Yusra, [1]Yempita, E., [2]Ainul, M. and [3]Nurul, H.

[1]*Department of Fisheries Resources Utilization, Faculty of Fisheries and Marine Science, Universitas Bung Hatta, Padang, West Sumatera, Indonesia-25133*
[2]*Department of Aquaculture, Faculty of Fisheries and Marine Science, Universiti Nahdlatul Ulama, Padang, West Sumatera, Indonesia-25133*
[3]*Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Kota Kinabalu, 88400 Sabah, Malaysia*

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Abstract

Fungi contamination of foods including raw and processed fish results in deterioration of food quality. The use of biological agents in the form of microbes containing antifungal activities is a promising solution and is essential to study. This study aimed to determine the antifungal activities of the lactic acid bacteria (LAB) isolated from "budu fish", a type of fermented dried fish against the growth of pathogenic fungi found in dried sepat (*Trichogaster trichopterus*). A total of six LAB isolates (B-1.6, B-1.8, B-1.14, B-2.1, B-2.3, and B-2.5) were used to identify the antifungal activities conducted with an overlay method. The LAB identification was carried out based on morphological and biochemical leading to the result that the six LAB isolates were *Lactobacillus* sp. and *Pediococcus* sp. bacteria. All of the LAB isolates formed inhibition zones against the growth of *Mucor* sp., *Aspergillus niger*, *Aspergillus fumigatus*, and *Penicillium nigricans* fungi. *Lactobacillus* sp. B-1.6 isolate had the highest antifungal activity, and through PCR identification, it was found that this bacterium was *Lactobacillus brevis* strain NCTC13768. *Lactobacillus brevis* strain NCTC13768 produced an antifungal chemical that caused morphological harm to *Aspergillus fumigatus* and *Aspergillus niger*. All of the isolates tested in this investigation are promising antifungals that could be employed to improve the safety of dried fish.

1. Introduction

The issues of food safety and availability, and also global nutrition, have been highly concerned, especially with the increasing human population and the impact of the COVID-19 pandemic (FAO, 2021). Food safety has been a global public health priority as 10% of people around the world get sick from foodborne diseases (Bumyut *et al*., 2021). Food safety is determined by the presence or absence of harmful components physically, chemically, or microbiologically. Microbiological hazards occur from pathogenic bacteria and toxins that are caused by food (Government Regulation of the Republic of Indonesia, 2019). More than 400,000 people are passing away every year due to the consumption of food contaminated with bacteria, viruses, parasites, and toxic chemicals (WHO, 2021). It is estimated that around 1.3 billion tons of food are wasted every year, including fresh and processed fish (FAO, 2019). The main factor causing this damage is the spoilage caused by microbes, including bacteria and fungi (Odeyemi *et al*., 2019; Pato *et al*., 2021).

Fungi are a major problem in every food chain, starting from the stages of post-harvesting, processing, and transporting, to storing (Davies *et al*., 2021). Most fungi, such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*, can produce mycotoxins, which can cause spoiling during the post-harvesting stage, resulting in economic consequences (Kizis *et al*., 2021). The types of fungi that often contaminate salted dried fish are *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., and *Fusarium* sp. (Olajuyigbe *et al*., 2014). The fungi discovered in the dried fish sold in Chennai city are *Aspergillus parasiticus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium* sp., *Cladosporium* sp., and *Euratium* sp. (Thiyagarajan *et al*., 2021). Some fungi found in the dried nile fish sold in Sudan are *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifara*,

*Corresponding author.
Email: *yusra@bunghatta.ac.id*

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Alternaria sp., and *Penicillium* sp. (Ahmed and Mahmoud, 2021).

On the other hand, the requirement for safe fish, particularly when it has the "halal" designation and is free of hazardous preservatives like borax and formalin, is critical. In recent years, there has been an increasing demand from consumers for food products free from preservatives with the use of antagonistic microorganisms (Dopazo *et al*., 2021). Lactic acid bacteria are the most common natural microorganisms used in fermentation products. Biopreservation using lactic acid bacteria (LAB) is currently the main alternative for food preservatives because it is able to produce antifungal metabolites (Shi and Knochel, 2021). There have been numerous researches on the use of LAB as an antifungals and that it has been applied to food products (Siedler *et al*., 2020; Li *et al*., 2020; Natasia *et al*., 2020; Quijal *et al*., 2020), but those derived from "budu fish" have never been conducted yet.

"Budu fish" is a fermented product produced in the coastal area of West Sumatera, which is commonly made from mackerel (*Scomberomorus guttatus*) and "talang" fish (*Chorinemus lyson* L.) (Yusra *et al*., 2013). Five types of bacteria have been isolated from these fermented products which are *Bacillus sphaericus*, *Bacillus polymyxa*, *Bacillus cereus* strain HVR22, *Bacillus pantothenticus* dan *Micrococcus lactis* (Yusra *et al*., 2014). From the fermented fish of "talang" fish from Aie Bangih Pasaman, the halophilic bacteria *Micrococcus* sp. was found (Fifendy and Biomed, 2017). *Bacillus cereus* strain HVR22 has the highest antimicrobial activity, and it can be used as a preservative for nile fish fillets (Yusra and Efendi, 2017). *Lactobacillus* sp. from "budu" can produce glutamic acid which is used as a feed supplement in broiler chickens (Maslami *et al*., 2018). It is also found in "budu" that lactic acid bacteria are capable of producing *Gammaamino butyric acid* (GABA) functioning as an anti-stress in broilers (Anggraini *et al*., 2019). Three isolates of yeast (*Saccharomyces* sp.), consisting of SC 11, SC 12, and SC 21, are found in "budu" which can be used as probiotics (Marlida *et al*., 2021). This study aimed to identify and analyze the antifungal activities of lactic acid bacterial isolates derived from "budu fish". As a result, it can be used to predict the quality decline in salted fish in order to improve food safety.

2. Materials and methods

The study was carried out at The Integrated Research Laboratory, Faculty of Fisheries and Marine Sciences, the University of Bung Hatta, West Sumatra, Indonesia, from January 2021 to August 2021.

2.1 Source of lactic acid bacteria

The samples used in this research were six LAB isolates from "budu fish" with the codes B-1.6, B-1.8, B-1.14, B-2.1, B-2.3, and B-2.5. The stock cultures were stored in the De Man Rogosa (MRS) broth containing 20% glycerol at -20^{o}C. To rejuvenate the LAB culture, one ml of bacterial isolate suspension was put into 10 mL (MRS) broth and incubated at 37°C for 24 hrs.

2.2 Identification of lactic acid bacteria

The LAB morphology and biochemistry identifications were conducted following the method of Krieg *et al*. (2010).

2.3 Salted fish sampling and preparation

The dried sepat (*Trichogaster trichopterus*) were bought in three traditional markets in Padang City, West Sumatera: Lubuk Buaya, Ulak Karang, and Raya. The sepat were put in a plastic bag, sealed, labelled, and brought to the Microbiology Laboratory of the Faculty of Fisheries and Marine Sciences, Bung Hatta University, Padang. The samples were stored at room temperature (28±2°C) for 4-7 days to allow fungal growth on the body surface.

2.4 Mycological analysis

The grown fungi were then isolated using Potato Dextrose Agar (PDA, difco) media at a temperature of 30^{o}C. A total of 1 g of sepat fish from each market was homogenized into 9 mL of sterile distilled water. After a serial dilution (10^{-7}), 1 mL of the sample culture was spread on the Potato Dextrose Agar (PDA) media which had been added with 40 g/mL chloramphenicol. The morphology of the growing fungal colonies included the color and size (CFU/g) after 5 days of incubation at room temperature (28±2°C) (Adesokan *et al*., 2016).

2.5 Fungal isolation and purification

A total of 10 mL cultures of each sample were spread on the sterilized PDA media and incubated at 28±2°C temperature for 5 days. Colonies with different morphology were re-subcultured on a fresh medium to obtain pure isolates and then stored at 4^{o}C on the PDA media. After being incubated for 5 days, different fungal species were identified based on the morphology of the colony, color, size, and texture. The pure isolates were then numbered and stored at -4^{o}C for further identification (John and Weber, 2007).

2.6 Determination of antifungal activity

The inhibitory activity of LAB isolates was determined by the overlay method as described with

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slight modifications (Muhialdin and Hasan, 2011). LAB was inoculated by straining a 2-cm-like line on MRSA and incubated aerobically at 37°C for 24 hrs. Agar was then coated with 10 mL PDA (40°C) containing 10^7 spore/mL of each fungal isolate and incubated aerobically at 28±2°C. After that, observations were conducted on the inhibition zones that were formed. Inhibitory activity was calculated based on the clear zone area that is formed by the following criteria: no inhibition (-), very weak inhibition (+), weak inhibition (++), and strong inhibition (+++).

2.7 Identification of lactic acid bacteria molecularly

The identification of LAB isolates was carried out molecularly based on partial genetic analysis on 16S rDNA. DNA extraction was conducted using the GES method, modified (Pitcher *et al*., 1989). The PCR amplification of 16S rDNA used 27F primers: 5'-AGA GTT TGA TCC TGG CTC AG-3' dan 1492 R:5'-GGT TAC CTT GTT ACG ACT T-3'(White *et al*., 1990). The purification of the PCR product was carried out by the PEG precipitation method (Hiraishi *et al*., 1995), and then it continued with the sequencing cycle. The primers used for sequencing were 27F: 5'-AGA GTT TGA TCC TGG CTC AG-3' dan 1492 R:5'-GGT TAC CTT GTT ACG ACT T-3'. The results of the sequencing cycle were re-purified by the ethanol purification method. The analysis of the nitrogen base sequence used an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The raw data from the sequencing was then trimmed and assembled using the BioEdit program (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) (Kimura, 1980; Kumar *et al*., 2018)

2.8 *Scanning Electron Microscopy*

Mycelium from *Aspergillus fumigatus* and *Aspergillus niger* was collected and fixed with 2.5% of glutaraldehyde at 4°C for 24 hrs. The next stage was PBS wash buffer as many as 3 times for 15 mins each. After fixation, dehydration was carried out using graded ethanol solutions (30%, 50%, 70%, 85% and 90%) for 15 mins, and was finally dehydrated twice with 100% ethanol for 20 mins. The samples were soaked in 100% isoamyl acetate twice for 20 mins each. After that, the specimen was dried and mounted on an aluminum stub with a double-stick, then plated with gold for further observation using a Hitachi S-750 SEM microscope (Xing *et al*., 2014).

3. Results and discussion

3.1 Identification of lactic acid bacteria

The LAB isolates identification was carried out based on morphological (Table 1) and biochemical (Table 2) characteristics. The observation of colony morphology included form, color, diameter, surface, consistency, Gram stain, and catalase test. The results of LAB characterization showed that the isolates were circular, cream colored, that the colony diameter was 0.5 – 2.0 mm, and that the surface was shiny and sticky (Figure 1). Based on the morphological and biochemical characteristics which were then matched with the identification key by Barrow and Feltham (1965), it was found that the six LAB isolates in "budu fish" were grouped into two (2) genera which were *Lactobacillus* (B -1.6, B-1.8, B-1.14, B-2.3 dan B-2.5) and *Pediococcus* (B-2.1). This finding was in line with the research by Liu *et al.* (2021) that the fermented fish products from China "suanyu" produced some bacteria from the genera of *Lactobacillus* with a percentage of 53.99%, *Tetragenococcus* 35.60%, and *Weissella* 4.10%. The major bacterium detected in Sumbawa's fermented shrimp products belonged to the *Lactobacillus* genus, with a proportion of 96.96 to 98.63% (Manguntungi *et al.,* 2020). Next, the isolation of LAB from the fermented product of "peda" fish led to the finding of *Lactobacillus plantarum, Lactobacillus curvatus, Lactobacillus murinus, and Streptococcus thermophilus* bacteria (Putra *et al.,* 2018). *Lactobacillus plantarum* and *Pediococcus pentosaceus* had successfully been isolated from "pekasam", a fermented fish product originating

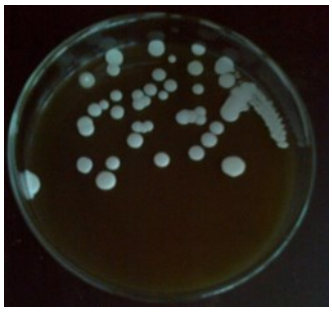

Figure 1. LAB colony morphology.

Table 1. Characteristics of LAB colony morphology of "budu".

Isolate Code	Colony Form	Colony Morphology			Cell Morphology	
		Edge	Color	Elevation	Cell Form	Gram
B-1.6	Circular	Flat	Yellowish white	Convex	Bacilli	Positive
B-1.8	Circular	Flat	Yellowish white	Convex	Bacilli	Positive
B-1.14	Circular	Flat	Yellowish white	Convex	Bacilli	Positive
B-2.1	Circular	Flat	Yellowish white	Convex	Bacilli	Positive
B-2.3	Circular	Flat	Yellowish white	Convex	Bacilli	Positive
B-2.5	Circular	Flat	Yellowish white	Convex	Bacilli	Positive

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Table 2. Characteristics of LAB colony from "budu" based on biochemical tests.

Characteristics	Isolate Code					
	B-1.6	B-1.8	B-1.14	B-2.1	B-2.3	B-2.5
Motility	-	-	-	+	-	-
Oxidase	-	-	-	-	-	-
Aerobic/anaerobic	A	A	A	A	A	A
Indole	-	-	-	-	-	-
Nitrate Reduction	-	-	-	-	-	-
TSIA	A/A	A/A	A/A	K/K	A/A	A/A
Glucose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Gas	-	-	-	-	-	-
Citric	-	-	-	-	-	-
Blood Agar	-	-	-	+	-	-
Pigmentation	Gray	Gray	Gray	Gray	Gray	Gray
Hemolysis	+	+	+	-	+	+
Urea	+	+	+	-	+	+
Mannitol	+	+	+	+	+	+
MR	-	-	-	+	-	-
VP	-	-	-	-	-	-
OF	-	-	-	-	-	-
Gelatin	-	-	-	+	-	-

from Malaysia (Muryany *et al*., 2017). *Staphylococcus*, *Micrococcus,* and *Bacillus* bacteria were found in "punti shidal" and "phase shidal", the fermented fish products originating from India (Majumdar *et al*., 2016). Meanwhile, *Pediococcus acidilactici* strain PB22 was the dominant bacterium and had the highest antimicrobial activity in "bekasam", a fermented product of three-spot gourami (*Trichopterus trichopterus*) from Banyuasin area, South Sumatra province, Indonesia (Melia *et al*., 2019). The three dominant types of lactic acid bacteria isolated from "plara", a fermented fish product from three Thai markets (khon kaen, kalasin and roi et), were *Tetragenococcus muriaticus, Haloanaerobium fermentans*, and *Lactobacillus rennini* (Rodpai *et al*., 2021). From the traditional Indian fish fermentation product "shidal", the bacteria of *Lactobacillus plantarum*, *P. pentosaceus*, *P. acidilactici*, *P. lolii*, *Enterococcus hirae*, *Enterococcus lactis*, *E. faecium*, and *E. faecalis* were found (Gupta *et al*., (2021).

3.2 Isolation and variety of fungi contaminating the dried fish

The morphological form and microscopic characteristics of the fungi isolates of dried sepat (*Trichopterus trichopterus*) can be seen in Table 3. Four fungi isolates found in the dried sepat sold in the three markets in Padang City were *Muco*r sp, *Aspergillus nig*er, *Aspergillus fumigatus*, and *Penicillium nigricans*, *Mucor* sp. and *Aspergillus niger* fungi were found in the dried sepat sold in Lubuk Buaya market; *Aspergillus fumigatus* was found in the dried sepat sold in Ulak Karang market; *Penicillium nigricans* and *Mucor* sp. were found in the dried sepat sold in Raya market. The finding of fungi in dried sepat was caused by the poor systems of processing, storage, and distribution to the consumers. This could be attributed to the storage conditions of dried fish which were usually placed in a humid place. In addition, fungal contamination in dried fish was also due to their stronger reproductive and diffusion capabilities. The spore production in the fungi made it easier for them to survive and attach to high-protein nutrient sources, one of which was dried fish. These findings were in line with the research of Deng *et al.* (2021) that three dominant fungi genera were found: *Aspergillus* sp*., Penicillium* sp. and *Fusarium* sp. in some dried fish species (*Hemibarbus maculatus*, *Pseudosciaena crocea*, *Lutjanus erythopterus*, *Thunnus thynnus*, *Scomberomorus niphonius*, *Eleutheronema tetradactylum*, *Trichiurus lepturus*) sold in the fish market in Zhanjiang, China. Furthermore, according to the research of Singapurwa *et al.* (2018), regarding the identification of the contaminant fungi in the dried lemuru (*Sardinella lemuru*) products, the species of *flavus*, *Aspergillus aculeatus*, *Aspergillus niger*, and *Aspergillus tubingensis* were found. Based on the research of Shamsan and Al-Jobory (2018), 26 species of mold in "wazef" (sun-dried) fish were found, including *Aspergillus* sp., *Rhizopus* sp., and *Penicillium* sp. According to Awe and Adejo (2018), the species of fungi frequently found in dried fish were *Penicillium* sp., *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp.,

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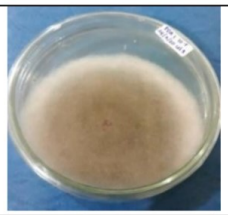
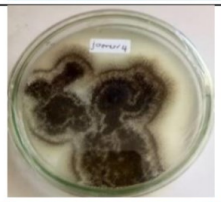
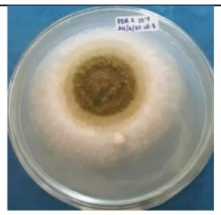
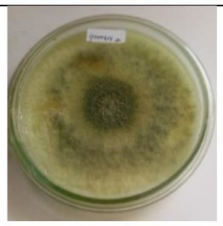
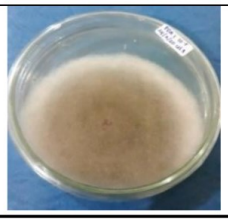
Table 3. Colony morphology and mold microscopic observation.

Sample Code	Traditional Market	Mold Type	Colony Morphology	Microscopic Observation
1	Lubuk Buaya	*Mucor* sp.	The colony color is white like cotton.	
		Aspergillus niger	The surface of the colony appears; the texture is smooth; the spores are blackish white; the color intensity increases as the older it becomes.	
2	Ulak Karang	*Aspergillus fumigatus*	The colony texture is like cotton or wool thread, and is in a granular form, the color is greenish gray.	
3	Raya	*Penicillium nigricans*	The colony color is yellowish green or slightly pale blue green, 1.5 cm in diameter, the colony is like cotton, the colony edge is uneven.	
		Mucor sp.	The colony color is white like cotton.	

Mucor sp., and *Rhizopus stolonifer*. The most commonly found fungi on the surface body of the sun-dried fish sold in the market were the genera of *Aspergillus* sp. (Nyamwaka *et al*., 2017). The results of this study were in line with the research of Ahmed and Mahmoud (2021) who succeeded in isolating the molds *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Alternaria* sp., and *Pencillium* sp. in the dried salted nile fish sold several areas in Sudan.

3.3 Antifungal spectrum

The inhibitory activity of LAB isolates against pathogenic fungi derived from dried sepat can be seen in Table 4. The LAB strength in inhibiting fungi is seen based on the zone of inhibition that arises around the bacterial colonies. The area of the inhibition zone was scored as follows: (-) no inhibition; (+) weak inhibition (≤ 0.5 mm); (++) moderate inhibition (0.6- 1.4 mm); (+++) strong inhibition (1.5- 2.4 mm). Most of the selected LAB isolates showed moderate (++) to strong (+++) antifungal effects against *Mucor* sp., *Aspergillus niger*, *Aspergillus fumigatus*, and *Penicillium nigricans*. The inhibition level of the fungal growth using the overlay method depicted that the isolates of *Lactobacillus* sp. B-1.6 showed the greatest antifungal activity (Figure 2). The inhibition strength on the fungal growth was because LAB was capable of producing various metabolites including lactic acid, acetic acid,

Table 4. Inhibitory activity of LAB isolates against fungal pathogens.

Code	Species	*Mucor* sp.	*Aspergillus niger*	*Aspergillus fumigatus*	*Penicillium nigricans*
B-1.6	*Lactobacillus* sp.	+++	+++	+++	+++
B-1.8	*Lactobacillus* sp.	++	+++	++	++
B-1.14	*Lactobacillus* sp.	+++	+++	++	++
B-2.1	*Pediococcus* sp.	++	+++	++	++
B-2.3	*Lactobacillus* sp.	++	+++	++	++
B-2.5	*Pediococcus* sp.	+++	+++	++	++

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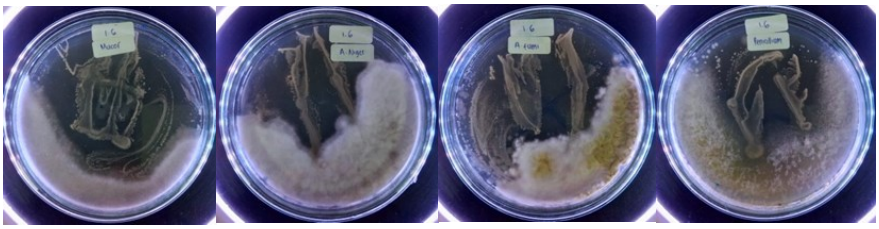

Figure 2. A clear inhibition zone of fungal growth was formed around the colonies of Lactobacillus sp. B-1.6 on MRS agar media.

propionic acid, and phenylactic acid (PLA). LAB was well-known for its antifungal activity, which was associated with the production of various compounds including organic acids, diacetyl, reuterin, hydrogen peroxide, phenylactic acid, bacteriocins, and cycle peptides (Crowley *et al*., 2013). The organic acids produced by LAB (acetic acid, caproic acid, formic acid, propionic acid, butyric acid, and n-valeric acid) synergized with each other to form antifungal compounds (Siedler *et al*., 2019). Furthermore, LAB was able to produce organic acids, short-chain fatty acids, hydrogen peroxide, reuterin, diacetyl, bacteriocins, and bacteriocin-like (Shehata *et al*., 2019). The cell-free supernatant from the *Lactobacillus brevis* MYSN105 bacterium could inhibit the growth of *Fusarium verticillioides* fungus which resulted from the role of the organic acids being produced (Mauro and Garcia, 2019). The results of this study were in line with the research of Stanzer *et al*. (2017) that the bacteria of *Lactobacillus brevis, P. pentosaceus, Weissella cibaria*, and *Lactobacillus farciminis* isolated from sourdough fermentation could significantly inhibit the growth of *Aspergillus niger* 357 and *Penicillium* sp. 505 fungi. The bacteria of *Lactobacillus brevis* 5M2 and *Lactobacillus buchneri* 6M1 were able to inhibit the growth of *Fusarium graminearum* (Paradhipta *et al*., 2020). In the traditional Indian fermented food products, one potential LAB isolate that could be used as an antifungal was found, which was *Lactobacillus brevis* MYSN105 (Somashekaraiah *et al*., 2021). The LAB isolates from foods originating from Turkey (cheese, whey, raw milk, boza, and yogurt) had various activities against the *Aspergillus candidus*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum*, *Mucor hiemalis*, *Ulocladium chartarum*, *Aspergillus niger,* and *Penicillium expansum bacteria* (Kanak and Yilmaz, 2020). After that, the *Lactobacillus plantarum*, *Lactobacillus fermentum*, and *Lactobacillus lactis* bacteria isolated from "kati", a fermented cereal-based food, were able to inhibit the growth of the Aspergillus flavus fungus (Gabriel *et al*., 2021).

3.4 Molecular identification of lactic acid bacteria

After conducting an antifungal test, it was found that one LAB isolate having the highest inhibitory activity was B-1.6 isolate. To determine the strain of *Lactobacillus* sp. B-1.6, identification using PCR was conducted based on the 16S rRNA gene sequence, followed by sequencing. This approach has become a common way of determining the relationship between bacteria and was widely used for identification purposes (Minervini *et al*., 2012). The *Lactobacillus* sp. B-1.6 isolate derived from "budu fish" was successfully amplified as indicated by the visible band measuring 1,500 bp. Based on the sequencing results which were analyzed using the BioEdit program and matched with reference data through the BLAST program on GenBank, a 99.78% homology was obtained with *Lactobacillus brevis* strain NCTC13768 (Figure 3). The results of this study were in line with the research on the LAB isolation in the fermented "budu" products which discovered that *Lactobacillus* sp. IB.9 had the potential to produce glutamic acid (Maslami *et al*., 2018). It was also found that LAB was capable of producing Gamma-aminobutyricacid (GABA) which was used as an antistress for broilers (Anggraini *et al*., 2019). From the fermented product of E-sarn sausage in Thailand, four dominant LAB were found, namely: *Lactobacillus brevis* KM495930.1, *Lactobacillus plantarum* MF369875.1, *Enterococcus durans* HQ603862.1, and *Pediococcus pentosaceus* NC-008525.1 (Wanangkarn *et al*., 2020). In "kimchi", Korean fermented vegetable, *Lactobacillus brevis* JCM 1059 was found (Sapalina and Retnaningrum, 2020). In "gajami sikhae", a fermented fish product originating from the Pohang area, Gyeongsangbuk-do, South Korea, *Lactobacillus brevis* GS1022 and *Pediococcus inopinatus* GS316 were discovered (Lim *et al*., 2020). In "kargi tulum", cheese in Turkey produced traditionally, *Lactobacillus brevis* KT38-3 was obtained, and it could function as probiotics (Hacioglu and Kunduhoglu, 2021). Another research on LAB which was able to inhibit the fungal growth was the *Lactobacillus brevis* 01M22 bacterium isolated from sourdough fermented bread which was the most effective strain in inhibiting the growth of three testing fungi: *Fusarium* sp., *Rhizopus* sp., and *Aspergillus* sp. (Petkova *et al*., 2021). Likewise, in the study of Di Biase *et al.*, (2014), it was stated that *Lactobacillus brevis* bacterium was effective in inhibiting *Aspergillus niger* growth on bread. *Lactobacillus brevis* SA-C12 was able to inhibit the growth of *Aspergillus carbonarius* (Li *et al.,* 2020).

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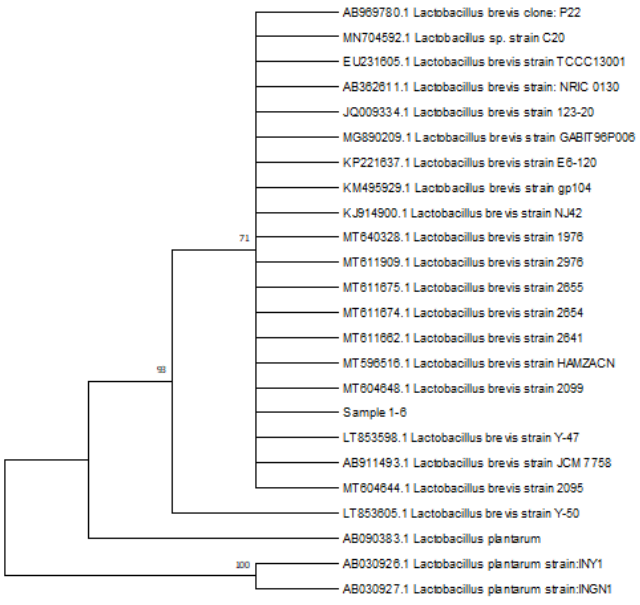

Figure 3. Phylogenetic tree of *Lactobacillus* sp. B-1.6 based on 16S rRNA sequences.

3.5 The effect of lactic acid bacteria on fungal morphology

Scanning Electron Microscope (SEM) was carried out to determine the effect of LAB *Lactobacillus brevis* strain NCTC13768 on the fungal morphology of *Aspergillus fumigatus* and *Aspergillus niger* (Figure 4). Changes in the morphological structure of the two fungi were seen in the figure. Based on the scan using SEM, the structural changes and morphological damage of *Aspergillus fumigatus* and *Aspergillus niger* could be seen after 5-day incubation. The total mass of mycelium produced was greatly reduced with the appearance of hyphae lysis which caused abnormal shape. This was due to hyphae rupture, and this caused loss of linearity. The peptide compound produced by the *Lactobacillus brevis* strain NCTC13768 bacterium caused obvious damage to the mycelium cell wall and a reduction in the size of the hyphae of *Aspergillus fumigatus* and *Aspergillus niger*. This was in line with the opinion of Rather *et al.* (2013) that there was damage to the morphology of the mycelium cell wall and a reduction in the size of the hyphae of *Aspergillus niger* treated with *Lactobacillus plantarum* YML007 bacteria after the scanning process was carried out using an electron microscope. Furthermore, Sangmanee and Hongpattarakere (2014) also analyzed the morphological damage of the *Aspergillus flavus* and *Aspergillus parasiticus* fungi due to exposure to *Lactobacillus plantarum* K35 bacterium. In another study, SEM was used to investigate the antifungal effect of *Lactobacillus pentosaceus* OCK 0979 bacterium on morphological changes which caused mycelium damage of *Alternaria brassicicola* (Lipinska *et al*., 2018). Microscopically, the peptide fraction produced by *L. plantarum* TE10 LAB could cause morphological damage to the *Aspergillus flavus* fungus (Muhialdin *et al*., 2020). Additionally, it was found through SEM and TEM that *Lactobacillus brevis* 8-2B bacterium could damage the hyphae, cell walls, cell membranes, and organelles of the *Aspergillus carbonarius* fungus (Li *et al*., 2021).

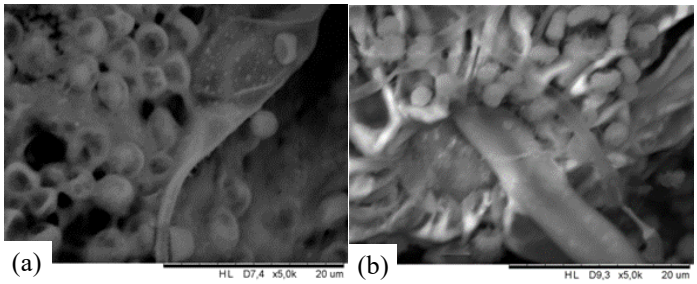

Figure 4. SEM analysis of antagonistic effects of *Lactobacillus brevis* B-1.6 on *Aspergillus fumigatus.* (a) and *Aspergillus niger* (b)

4. Conclusion

The six LAB isolates that were identified from "budu fish" were *Lactobacillus* sp. and *Pediococcus* sp., according to the investigation. Four types of fungi were discovered based on the fungal isolation and identification of dried sepat sold in the three markets: *Mucor* sp., *Aspergillus niger*, *Aspergillus fumigatus*, and *Penicillium nigricans*. The four different species of fungi were all inhibited by all of the LAB isolates. The antifungal activity of *Lactobacillus* sp. B-1.6 isolate was the highest, and PCR identification revealed that this bacterium was *Lactobacillus brevis* strain NCTC13768. *Lactobacillus brevis* strain NCTC13768 developed an antifungal chemical that caused morphological harm to *Aspergillus fumigatus* and *Aspergillus niger* fungus.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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