



## Selection of potential lactic acid bacteria from fish intestine of mackerel (*Rastrelliger kanagurta*) from Lembata Regency, East Nusa Tenggara, Indonesia

Helena Daten\*, Tri Ardyati and Yoga Dwi Jatmiko

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Veteran Street, Malang, East Java, 65145 Indonesia.  
Email: [helenadaten061095@gmail.com](mailto:helenadaten061095@gmail.com)

Received 17 September 2021; Received in revised form 31 August 2022; Accepted 6 September 2022

### ABSTRACT

**Aims:** The aim of this study was to screen lactic acid bacteria (LAB) isolates with probiotic properties for tilapia (*Oreochromis niloticus*) from the intestines of mackerel and to analyze its ability to produce hydrolase enzymes (amylase, protease, cellulase and lipase).

**Methodology and results:** Ten samples of mackerel were collected from the Fish Auction Place, Lewoleba, Nubatukan District, Lembata Regency, East Nusa Tenggara Province, Indonesia. The process for screening were antimicrobial activity, pH tolerance test of 1, 3 and 5, bile salt concentrations of 2.5%, 5% and 7.5%, and water salinity (0.5%, 3.5% and 6.5%). The autoaggregation and semi-qualitative test of hydrolase enzymes were also carried out. In total, 21 LAB isolates were derived from mackerel intestines. Isolates that have the stronger ability to inhibit the pathogen *Aeromonas hydrophila* ATCC 7966 are KBP 3.3, KBP 3.3.1 and KBP 6.3, while KBP 1.3, KBP 1.1.1 and KBP 3.3.1 were able to inhibit *Streptococcus agalactiae* ATCC 13813. The LAB isolates that survived at the tested pH were KBP 3.3.1, KBP 3.3, KBP 1.1.1 and KBP 1.3. Results also suggest that the four isolates were tolerant to bile salt concentrations and water salinity with good survival rates (>94.7%) and had proteolytic, lipolytic and cellulolytic activities. The isolates KBP 3.3, KBP 3.3.1 and KBP 1.1.1 were identified as *Weissella confusa* 6250, while the isolate KBP 1.3 was identified as *W. confusa* C5-7.

**Conclusion, significance and impact of study:** The characteristics of four selected isolates indicate their potential as a probiotic. These potential probiotic isolates can be applied directly to fish by utilizing microencapsulation technology or adding to the feed.

**Keywords:** Lactic acid, Lembata regency, mackerel, probiotic, tilapia

### INTRODUCTION

Fishing in Indonesia from year to year has increased and each region has a different fishery potential. An example is Lembata Regency, East Nusa Tenggara (NTT) Province. The type of catch fish mainly produced in Lembata Regency was mackerel (*Rastrelliger kanagurta*). Its reported production was 206.03 million tons at the end of 2016 (Central Bureau of Statistics of NTT, 2016). Mackerel is one of the small pelagic fish scattered throughout the Indonesian sea and live in groups that were easily caught by fishermen (Prahadina *et al.*, 2015) and it's the most consumed fish species by the people of Lembata. High fish yield must support high community demand. Usually, the number of catches was influenced by weather factors such as rain and the wind. Therefore, one of the efforts to meet community demand is freshwater aquaculture (Abubakar *et al.*, 2019).

The most widely cultivated freshwater fish is tilapia (*O. niloticus*) (Dawood *et al.*, 2019). Tilapia is important for cultivation because of its distinctive meat taste and very fast growth and development rate. Intensive tilapia cultivation causes stress to the fish, lowering their immune system and making them susceptible to disease. Infectious diseases are usually caused by pathogens such as *A. hydrophila* and *S. agalactiae*, which cause septicemia and hemorrhage in tilapia. One of the efforts to solve this problem is the application of probiotics that are safe for the host and the surrounding environment. (Iwashita *et al.*, 2015; Tan *et al.*, 2019; Yilmaz, 2019; Dawood *et al.*, 2020; Kuebutornye *et al.*, 2020; Mohammadi *et al.*, 2020). The development of intensive and semi-intensive cultivation businesses must be supported by quality feed. Quality feed for fish is usually added with probiotics as a supplement to improve fish health.

\*Corresponding author

The Joint Food and Agricultural Organization/World Health Organization define probiotics as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. The microbiota communities play a role in the maintenance of human and animal physiology (Collado *et al.*, 2009; Fernandez-Pacheco *et al.*, 2018). The possible sources for probiotics could be the indigenous microbiota of the gut, as any microorganism could be a possible candidate as a probiotic since all contribute, either alone or in synergy, to the generation of benefits (Suárez, 2015).

Probiotics are generally derived from members of the LAB. Therefore, to increase the utilization of mackerel, which is widely produced in Lembata Regency, NTT, Indonesia, not only as food but also as a source of isolation of LAB, which has the potential as a probiotic. To be included in the probiotic group, a microorganism has to overcome criteria to survive in the host organism, such as changes in pH when passing through the gastrointestinal tract, exposure to gastric juice, enzymes and bile salt tolerance (Fernandez-Pacheco *et al.*, 2018). Therefore, if LAB is isolated from mackerel (*Rastrelliger kanagurta*) and applied to freshwater fish such as tilapia, the LAB must be tolerant to the physiological and environmental of tilapia. Therefore, this study aimed to screen lactic acid bacteria, which has the potential as a probiotic for tilapia from the intestines of mackerel. Probiotics obtained will be applied directly to tilapia and the outcomes are environmentally friendly and sustainable aquaculture.

## MATERIALS AND METHODS

### Sample collection and preparation

Mackerel was obtained from a fish auction place, Lewoleba Impres Market, Lembata Regency, NTT province, Indonesia. Mackerel length ranged from 18-19 cm, while fish weight ranged from 76.5 to 77.2 grams. The isolation of LAB and probiotic screening were carried out in the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. The bacterial pathogen strains of *A. hydrophila* ATCC 7966 was obtained from the Fish Disease Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya and *S. agalactiae* ATCC 13813 was obtained from the Research Center for Freshwater Aquaculture Fisheries, Sempur, Bogor, West Java, Indonesia.

A sample of ten mackerel fish was composited. Fish intestines were washed, mashed and homogenized with a NaCl concentration of 0.85%. Isolation was carried out by pour plate method into deMan, Rogosa and Sharpe (MRS) agar containing 1% CaCO<sub>3</sub>. It was used for the initial selection and purification of LAB because it is alkaline and had the ability to neutralize the acid produced by LAB and a clear zone characterizes it. After that, it was incubated at 37 °C for 48 h. Colonies that grew and formed a clear zone were purified using the spread plate method. The pure isolates obtained were subcultured on slanted agar media and stored at 4 °C.

The LAB isolates were characterized with Gram stain, cell size, cell shape and catalase test (Kavitha *et al.*, 2018).

### Screening of antimicrobial activity

The aim of the antimicrobial activity test was to determine the ability of LAB to inhibit pathogenic bacterial strains that cause infectious diseases in tilapia and freshwater fish in general. The method used to test the antimicrobial activity uses the well-diffusion method in triplicates. The pathogens *A. hydrophila* ATCC 7966 and *S. agalactiae* ATCC 13813 were grown in Trypticase Soy Broth (TSB). The number of cells for each culture of pathogenic bacteria and LAB isolates used for this test was 10<sup>7</sup> CFU/mL. After that, 0.1 mL of pathogen isolate was inoculated into Trypticase Soy Agar (TSA) media and then were spread plate and left for 10 min. Afterward, well were made using a cork borer (5 mm diameter) and 80 µL of culture for LAB isolate was added to wells. Afterward, it was incubated at 37 °C for 24 h and the clear zone formed was measured using a vernier caliper. The inhibition index was measured using the following equation 1 (Feng *et al.*, 2016).

$$\text{Inhibitory index} = (\text{Diameter of clear zone} - \text{Diameter of well}) / \text{Diameter of well} \quad (1)$$

### *In vitro* tests for probiotic properties: Tolerance to low pH, bile salt and water salinity

The number of cells used in this test was 10<sup>7</sup> CFU/mL. The LAB inoculum was added 10% in MRS broth media with variations in pH 1, 3 and 5, variations in bile salts 2.5%, 5% and 7.5%, and variations in water salinity 0.5%, 3% and 6.5%. Preparation of pH was carried out by adjusting the MRS broth medium by adding 0.1 M NaOH or 0.1 M HCl little by little and measured using a pH meter. The bile salt test was carried out by adding MRS broth media with oxgall media according to the required concentration. Preparation of water salinity test was carried out by adding MRS broth with NaCl according to the required salt concentration. All tests were performed in triplicates, and incubation was done at 37 °C for 24 h (Ramesh *et al.*, 2015). Sampling was carried out at 4 and 24 h. Afterward, the cell counting was done using hemocytometry method (Allameh *et al.*, 2012) and the survival rate of LAB isolates was measured using the following equation 2.

$$\text{Survival rate (\%)} = (\text{Log } N_1 / \text{Log } N_0) \times 100 \quad (2)$$

Where N<sub>1</sub> is the final cell amount and N<sub>0</sub> is the initial cell amount (Rajoka *et al.*, 2018).

### Auto-aggregation property

The LAB isolates were incubated at 37 °C for 24 h. The LAB isolates were centrifuged at 6000 rpm for 10 min and the pellets were suspended in saline solution to 15 mL. The suspension was taken 2 mL to measure optical

density (OD) at a wavelength of 600 nm. Afterwards, the suspension was left for 60 min at 30 °C. After 60 min, the suspension was centrifuged again at 3000 rpm for 2 min and the supernatant was separated from the pellet to measure OD at a wavelength of 600 nm. This test was performed in triplicates. The auto-aggregation percentage was measured using the following equation 3.

$$\text{Auto-aggregation (\%)} = \left[ \frac{(\text{OD}_0 - \text{OD}_{60})}{\text{OD}_0} \right] \times 100 \quad (3)$$

Where OD<sub>0</sub> is the OD value for 0 h and OD<sub>60</sub> is the OD value after 60 min of incubation (Padmavathi *et al.*, 2018).

### Semi-qualitative of enzymatic activity test

Enzyme activity test using paper disc diffusion method in triplicates. The activity of amylase, protease, lipase and cellulase enzymes was detected by placing paper discs in LAB inoculum for 15 min. Afterwards, the discs were placed on the test medium and incubated at 37 °C for 48 h. The amylolytic activity test used starch agar media with the composition of peptone, bacto agar, NaCl, MgSO<sub>4</sub>, CaCl<sub>2</sub>, yeast extract and starch. After that, the clear zone formed was observed by adding Gram's iodine as a qualitative detector of the amylase enzyme. Test of proteolytic activity using skim milk media (Padmavathi *et al.*, 2018). Test of lipolytic activity using the composition of peptone, NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O, agar, Tween-80 2.5% and sterile olive oil 5% (Bestari and Suharjono, 2015). Test of cellulolytic activity using carboxymethyl cellulose (CMC) media. The composition of the CMC was 1% yeast extract, KH<sub>2</sub>PO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub>, MgSO<sub>4</sub>, CaCl<sub>2</sub>, FeSO<sub>4</sub> and agar (Habbale *et al.*, 2019). After incubation, 1% Congo red was added and left for 30 min. Enzyme activity was indicated by the clear zone formed. The enzyme hydrolysis index was measured by dividing the diameter of the clear zone by the diameter of the paper disc (Bestari and Suharjono, 2015).

### Molecular identification

The DNA of the probiotic isolate was extracted using Zymo-Spin™ Lysis Kit by following the manufacturer's instructions. The DNA was amplified using 16S rDNA universal primers, namely 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492r (5'-GGT TAC CTT CTG ACT ACT T-3') with an expected DNA size of 1500 bp (Chen *et al.*, 2015). Composition of PCR reaction: go tag green (25 µL), forward primer 27f (2 µL), reverse primer (2 µL), DNA template (5 µL) and DdH<sub>2</sub>O (16 µL). The PCR reaction was run with an initial denaturation at 94 °C for 5 min (1 cycle), followed by denaturation at 94 °C for 1 min, annealing at 54 °C for 30 sec and extension at 72 °C for 1 min (35 cycles), with a final extension at 72 °C for 7 min (1 cycle). The 16S rDNA amplicon size was confirmed using agarose gel electrophoresis (1.5%). The phylogenetic tree was constructed using MEGA 6 software for windows based on Neighbor-Joining algorithm and Tamura-Nei model with a bootstrap of 1000 (Ng *et al.*, 2015).

### Data analysis

All assays (antimicrobial activity, tolerance to low pH, bile salt, water salinity, auto-aggregation and enzymatic activity) were conducted in triplicates. The antimicrobial activity and auto-aggregation data were analyzed using a one-way analysis of variance (ANOVA) and Tukey's HSD test ( $p < 0.05$ ). Meanwhile, the pH, bile salt, salinity and enzymatic activity data were analyzed using two-way ANOVA and Tukey's HSD test ( $p < 0.05$ ). All statistical analyses were completed using SPSS for windows version 16.0.

## RESULTS AND DISCUSSION

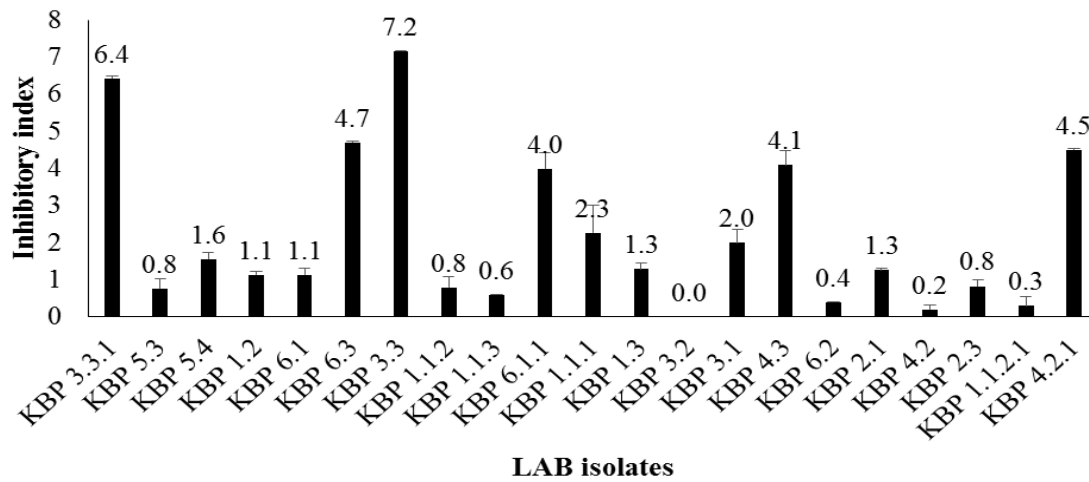
### Morphology of lactic acid bacteria cell

The average number of LAB cells mackerel's intestines is  $7 \times 10^6$  CFU/g. In total, 21 isolates of LAB were isolated. Cell characterization of these isolates was Gram positive and catalase negative. There were ten isolates of LAB in the form of cocci with a cell size of 1 µm and 11 isolates of bacilli with cell sizes ranging from 2-3 µm. Isolates were Gram positive and catalase negative because LAB does not produce the enzyme catalase (Carr *et al.*, 2002). This is supported by previous research; namely, the characteristics of LAB isolated from the intestines of rainbow trout (*Oncorhynchus mykiss*) are Gram-positive, bacillus or cocci shaped, non-spore-forming, nonmotile and catalase negative (Fečkaninová *et al.*, 2019). The abundance of Gram-positive bacteria in marine fish was influenced by the habitat of the fish, the body parts of the fish and the number of samples used (Al Bulushi *et al.*, 2010).

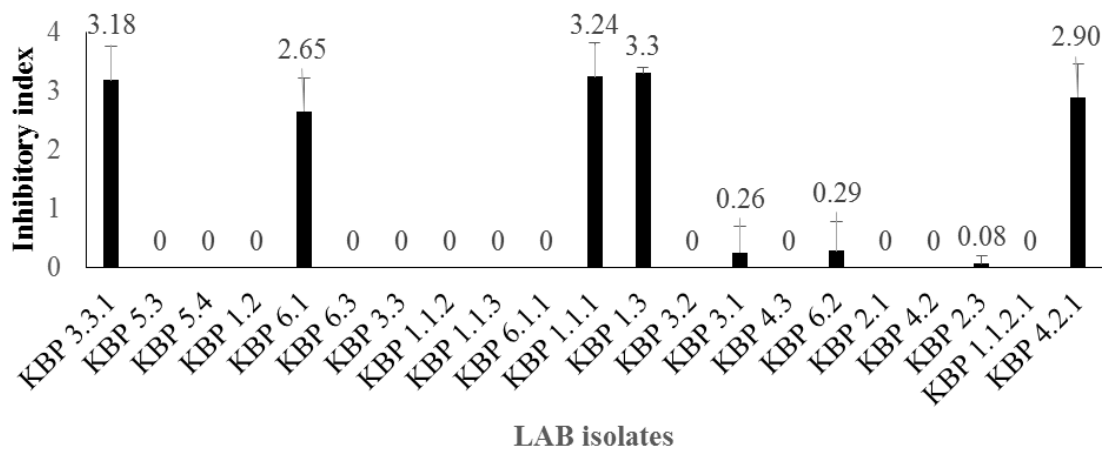
### Antimicrobial activity

Based on the results suggest that there were six isolates that were significantly different in inhibiting the pathogen *A. hydrophila* ATCC 7966. The isolates were KBP 3.3, KBP 3.3.1, KBP 6.3, KBP 4.2.1, KBP 4.3 and KBP 6.1.1 with inhibition indices of 7.16, 6.43, 4.7, 4.5, 4.1 and 4, respectively (Figure 1). The range of inhibition zones of LAB strains was <11 mm or an inhibitory index of <2.2 (no inhibitory activity), 11-16 mm or an inhibitory index of 2.2-3.2 (moderate inhibitory activity), 17-22 mm or an inhibitory index of 3.4-4.4 (strong inhibitory activity) and >22 mm or an inhibitory index of >4.4 (strongest inhibitory activity) (Feng *et al.*, 2019). Lactic acid bacteria have inhibitory activity if the inhibition category is moderate, strong and strongest. Therefore, isolates KBP 3.3, KBP 3.3.1, KBP 6.3, KBP 4.2.1 and KBP 4.3 were isolates with the strongest inhibitory activity, while isolates KBP 4.3 and KBP 6.1.1 were isolates that had strong inhibition.

Another ability of LAB is to inhibit the pathogen *S. agalactiae* ATCC 13813. The results of five isolates were significantly different ( $p < 0.05$ ) in inhibiting these pathogens. The isolates KBP 1.3, KBP1.1.1, KBP 3.3.1, KBP 6.1 and KBP 4.2.1, were with inhibition indexes of 3.3, 3.24, 3.18, 2.65 and 2.89, respectively (Figure 2). In



**Figure 1:** Antimicrobial activity of LAB against *Aeromonas hydrophila*. The notation on the graph shows a Tukey's HSD test ( $p < 0.05$ ), there is an effect of LAB isolates on the pathogen inhibitory index. Data was expressed as the mean from three replicates of each isolate, while the error bar indicates its standard deviation.



**Figure 2:** Antimicrobial activity of LAB against the pathogen *Streptococcus agalactiae*. The notation on the graph shows a Tukey's HSD test ( $p < 0.05$ ), there is an effect of LAB isolates on the pathogen inhibitory index. Data was expressed as the mean from three replicates of each isolate, while error bars indicate the standard deviation for the respective isolate.

addition, there was one isolate that was able to inhibit both pathogens with the highest inhibition index, namely KBP 3.3.1 (Figures 1 and 2). If the diameter of the inhibition zone was  $>13$  mm (inhibition index  $>2.6$ ), then the activity was classified as very strong; if the diameter of the inhibition zone was 10-13 mm (inhibition index 2-2.6) then the activity was classified as strong; the diameter of the inhibition zone is 5-10 mm (inhibition index 1-2) then the activity is classified as moderate; and if the diameter of the inhibition zone is  $<5$  mm (inhibition index  $<1$ ) then it was considered as no inhibitory activity (Chen *et al.*, 2019). Based on the inhibition index, the five isolates from the study were isolates that had very strong inhibitory activity (Figure 2). Compared with previous studies, the results of this study explained that five isolates were better at inhibiting pathogenic strains because their inhibition index was higher.

The LAB isolates produce organic acids such as lactic acid, which can be bactericidal. These organic acids are present in an undissociated form; thus, they can be lipophilic and can move into the cell membrane. Organic acids cause the pH in cells to be low. It can change the permeability of cell membranes which causes the transport system of nutrients such as compounds and ions in pathogenic bacteria to be destroyed. This can cause the cell to be damaged, followed by the disruption of the synthesis of the components that make up the cell wall, thus the cell wall can be lysis (Alakomi *et al.*, 2001; Allameh *et al.*, 2012; Alonso *et al.*, 2019). Moreover, some of the LAB isolates that have the ability to inhibit *A. hydrophila* are *Lactobacillus confusus* (9 mm), *L. brevis* (11.33 mm), *Lactococcus lactis* (9.33 mm) and *W. cibaria* (9.33 mm) (Mouriño *et al.*, 2016). *Aeromonas hydrophila* can be inhibited by

**Table 1:** Tolerance of lactic acid bacteria to pH 1, 3 and 5.

LAB isolates	Survival rate (%)					
	pH 1		pH 3		pH 5	
	4 h	24 h	4 h	24 h	4 h	24 h
KBP 3.3.1	95.4 ± 0.2 <sup>Ac</sup>	95.2 ± 0.12 <sup>Ac</sup>	100 ± 0 <sup>b</sup>	99 ± 1.5 <sup>b</sup>	100 ± 0 <sup>Aa</sup>	100 ± 0 <sup>Aa</sup>
KBP 6.3	56.9 ± 0.3 <sup>Abc</sup>	87.3 ± 0.52 <sup>Abc</sup>	92 ± 0.3 <sup>b</sup>	100 ± 2.9 <sup>b</sup>	100 ± 0 <sup>Aa</sup>	100 ± 0 <sup>Aa</sup>
KBP 3.3	94.7 ± 0.2 <sup>Ac</sup>	93.6 ± 1.6 <sup>Ac</sup>	100 ± 0 <sup>b</sup>	99 ± 2.7 <sup>b</sup>	98 ± 2.2 <sup>Aa</sup>	99 ± 0.7 <sup>Aa</sup>
KBP 6.1.1	91.5 ± 0.1 <sup>Ac</sup>	93.8 ± 1.4 <sup>Ac</sup>	100 ± 0.2 <sup>b</sup>	100 ± 0 <sup>b</sup>	100 ± 0 <sup>Aa</sup>	100 ± 0 <sup>Aa</sup>
KBP 4.3	56.1 ± 0.3 <sup>Aab</sup>	26.3 ± 0 <sup>Aab</sup>	93 ± 1.3 <sup>b</sup>	79 ± 0.9 <sup>b</sup>	100 ± 0 <sup>Aa</sup>	100 ± 0 <sup>Aa</sup>
KBP 4.2.1	82.9 ± 0.8 <sup>Abc</sup>	82.9 ± 0 <sup>Abc</sup>	35 ± 0.4 <sup>a</sup>	34 ± 2 <sup>a</sup>	99 ± 1.3 <sup>Aa</sup>	100 ± 0 <sup>Aa</sup>
KBP 6.1	20 ± 0 <sup>Aa</sup>	0.0 ± 0 <sup>Aa</sup>	100 ± 0 <sup>b</sup>	91 ± 5.2 <sup>b</sup>	100 ± 0 <sup>Aa</sup>	100 ± 0 <sup>Aa</sup>
KBP 1.1.1	95.8 ± 1 <sup>Ac</sup>	98.3 ± 2.3 <sup>Ac</sup>	95 ± 0.2 <sup>b</sup>	100 ± 0 <sup>b</sup>	99 ± 1.3 <sup>Aa</sup>	99 ± 1.4 <sup>Aa</sup>
KBP 1.3	100 ± 0 <sup>Ac</sup>	100 ± 0 <sup>Ac</sup>	100 ± 0.4 <sup>b</sup>	99 ± 1.2 <sup>b</sup>	100 ± 0 <sup>Aa</sup>	100 ± 0 <sup>Aa</sup>

The notation at pH 3 showed a Tukey's HSD test ( $p < 0.05$ ), namely, there was an interaction between LAB isolates and incubation time that affected the survival rate. Capital notations at pH 1 and 5 are notations to indicate the effect of LAB isolates on survival rate, while lowercase notations indicate the effect of incubation time on survival rate. The notation at pH 1 showed a Tukey's HSD test ( $p < 0.05$ ), while at pH 5 there was no significant ( $p > 0.05$ ).

*L. pentosus* AJ7 and *W. confusa* AJ79 bacteria (both inhibition zone diameter is 24 mm) (Dubey and Jeevaratnam, 2015). Some of *Weissella* genera that inhibit the growth of *A. hydrophila* pathogens are *Weissella hellenica* BCC 7293, *W. cibaria* 110, *Weissella paramesenteroides* DFR-8, *W. confusa* CP3-1 (Chavasirikunton *et al.*, 2007; Sriannual *et al.*, 2007; Pal and Ramana, 2010; Masuda *et al.*, 2011; Leong *et al.*, 2013; Chen *et al.*, 2014; Woraprayote *et al.*, 2015) and *Weissella helenica* D1510 (Chen *et al.*, 2014). The pathogen *S. agalactiae* can also be inhibited by *L. lactis* subsp. *lactis* CRL 1655 (Espeche *et al.*, 2009), *Enterococcus faecium* BNM58, *W. cibaria* BNM69, *Enterococcus faecalis* SMF10, *E. faecium* SMA1, *Leuconostoc mesenteroides* subsp. *cremoris* (*Lc. cremoris*) SMM69 (Muñoz-Atienza *et al.*, 2013), *E. faecalis* and *Weissella* sp. (Shahid *et al.*, 2017).

#### **In vitro tests for probiotic properties: Tolerance to low pH, bile salt and water salinity**

##### *Tolerance to low pH*

The gastric pH range of tilapia is between 1.5-5.8 (Pinpimai *et al.*, 2015; Zidni *et al.*, 2017; Kavitha *et al.*, 2018), while Zidni *et al.* (2017) and Kavitha *et al.* (2018) reported that the intestinal pH range of tilapia is between 6.9-7. The tolerance of LAB to pH variations can be seen in Table 1. As highlighted in table 1, pH 1 and 5 showed no interaction between isolates and incubation time ( $p < 0.05$ ). Incubation time at 4 h and 24 h was not significantly different ( $p > 0.05$ ), while between LAB isolates, there was a significant difference ( $p < 0.05$ ). The KBP 1.3 (survival rate above 100%) isolate can survive at pH 1 for 24 h. Other isolates such as KBP 3.3.1, KBP 6.3, KBP 3.3, KBP 6.1.1, KBP 4.3, KBP 4.2.1 and KBP 1.1.1 can survive above 50% for 4-24 h (Table 1). Previous research conducted by Maragkoudakis *et al.* (2006) found that LAB that survived at pH 1 were from the genus *Lactobacillus*. Lactic acid bacteria survive at low pH by

releasing lactic acid and protons out of cells to keep their cell membranes from being damaged (Vijayakumar *et al.*, 2015).

At pH 3, there was an interaction between incubation time and LAB isolates. Some LAB isolates maintained survival rates above 95% for 24 h. The isolates were KBP 3.3.1, KBP 3.3, KBP 6.1.1, KBP 1.3 and KBP 1.1.1. Meanwhile, all LAB isolates tolerated pH 5 with a 100% survival rate for 24 h (Table 1) and it was not significantly different ( $p > 0.05$ ). The resistance of LAB isolates to acid was due to the bacterial isolates being able to maintain the pH in the cytoplasm to be more alkaline than the extracellular pH. Bacteria not resistant to acidic conditions cannot maintain the pH in their cells, resulting in damage to cell membranes and intracellular components of bacteria that cause lysis. Some of LAB tolerant to pH 3 are *W. confusa* DD\_A7 (Dey *et al.*, 2019), *L. buchneri* (Wulandari *et al.*, 2020) and *W. confusa* YM5Y (Wang *et al.*, 2020). Lactic acid bacteria that tolerate pH 5 are *W. confusa* MD1, *W. cibaria* MD2 (Lakra *et al.*, 2020), *Pediococcus pentosaceus* (Jaafar *et al.*, 2019) and *L. mesenteroides* (Allameh *et al.*, 2012). Moreover, *Enterococcus faecalis* MAA1 and *L. lactis* W3-15 can tolerate pH 3 and 5 (Kong *et al.*, 2020).

##### *Bile salt tolerance*

Tilapia and freshwater fish generally have bile salt concentrations ranging from 1-10% (Pinpimai *et al.*, 2015; Kavitha *et al.*, 2018). Five LAB isolates, namely KBP 3.3.1, KBP 3.3, KBP 6.1.1, KBP 1.3 and KBP 1.1.1 were tested for bile salt tolerance. The results in Table 2 show that there was no interaction between incubation time and LAB isolates at bile salt concentrations of 2.5% and 7.5% ( $p > 0.05$ ). The five isolates had a survival rate that was not significantly different ( $p > 0.05$ ) at a bile salt concentration of 2.5%. The KBP 6.1.1 did not grow at a concentration of 7.5% for 24 h. At 5% concentration, there was an interaction between LAB isolates and incubation time ( $p < 0.05$ ). The survival rates of the five LAB isolates

**Table 2:** Tolerance of lactic acid bacteria to bile salt concentrations of 2.5%, 5% and 7.5%.

LAB isolates	Survival rate (%)					
	2.5%		5%		7.5%	
	4 h	24 h	4 h	24 h	4 h	24 h
KBP 3.3.1	96 ± 7.8 <sup>Aa</sup>	97 ± 5 <sup>Aa</sup>	101 ± 0.2 <sup>c</sup>	101 ± 0.3 <sup>c</sup>	99.4 ± 0.9 <sup>Ab</sup>	98.5 ± 1.5 <sup>Ab</sup>
KBP 3.3	97 ± 1.2 <sup>Aa</sup>	103 ± 1.1 <sup>Aa</sup>	99.6 ± 0.6 <sup>bc</sup>	100.9 ± 0.7 <sup>c</sup>	99.8 ± 0.1 <sup>Ab</sup>	99.5 ± 0.1 <sup>Ab</sup>
KBP 6.1.1	97 ± 1 <sup>Aa</sup>	94 ± 1.6 <sup>Aa</sup>	102.5 ± 0.7 <sup>c</sup>	97.6 ± 1.4 <sup>ab</sup>	0 ± 0 <sup>Aa</sup>	0 ± 0 <sup>Aa</sup>
KBP 1.1.1	102 ± 1.4 <sup>Aa</sup>	100 ± 0 <sup>Aa</sup>	99.2 ± 1.4 <sup>bc</sup>	96.6 ± 1.1 <sup>a</sup>	99.6 ± 0.6 <sup>Ab</sup>	99.1 ± 1.5 <sup>Ab</sup>
KBP 1.3	101 ± 0.6 <sup>Aa</sup>	100 ± 0 <sup>Aa</sup>	105.1 ± 1 <sup>c</sup>	109.3 ± 1.2 <sup>c</sup>	96.6 ± 5.8 <sup>Ab</sup>	97.1 ± 5.1 <sup>Ab</sup>

The notation at a concentration of 5% showed a Tukey's HSD test ( $p < 0.05$ ), namely, there was an interaction between LAB isolates and incubation time that affected the survival rate. Capital notation at concentrations of 2.5 and 7.5% are notations to indicate the effect of LAB isolates on survival rate, while lowercase notation indicates the effect of incubation time on survival rate. The notation at a concentration of 2.5% bile salts showed no significant difference between isolates at  $p > 0.05$ , while the notation at a concentration of 7.5% showed a significant difference between isolates at  $p < 0.05$ .

**Table 3:** Tolerance of lactic acid bacteria to water salinity of 0.5%, 3.5% and 6.5%.

LAB isolates	Survival rate (%)					
	0.5%		3.5%		6.5%	
	4 h	24 h	4 h	24 h	4 h	24 h
KBP 3.3.1	113.1 ± 0.7 <sup>c</sup>	113.9 ± 0.5 <sup>c</sup>	103.6 ± 0.7 <sup>Aa</sup>	106.4 ± 0.2 <sup>Aa</sup>	101.5 ± 0.4 <sup>Aa</sup>	99.2 ± 0.4 <sup>Aa</sup>
KBP 3.3	111.9 ± 0.8 <sup>c</sup>	113.4 ± 0.8 <sup>c</sup>	102.9 ± 3 <sup>Aa</sup>	108.1 ± 0.2 <sup>Aa</sup>	100.9 ± 0.2 <sup>Aa</sup>	107.1 ± 0.3 <sup>Aa</sup>
KBP 1.1.1	94 ± 0.1 <sup>a</sup>	97 ± 0.6 <sup>b</sup>	103.5 ± 2.1 <sup>Aa</sup>	106.8 ± 0.4 <sup>Aa</sup>	97.1 ± 0.3 <sup>Aa</sup>	103.4 ± 0.6 <sup>Aa</sup>
KBP 1.3	95 ± 1 <sup>a</sup>	97.5 ± 0.9 <sup>b</sup>	100.8 ± 0.3 <sup>Aa</sup>	106 ± 0.2 <sup>Aa</sup>	98.6 ± 0.7 <sup>Aa</sup>	98.1 ± 3.3 <sup>Aa</sup>

The notation at 0.5% salinity showed a Tukey's HSD test ( $p < 0.05$ ), namely, there was an interaction between LAB isolates and incubation time that affected the survival rate. Capital notation at 3.5% and 6.5% salinity indicates the effect of LAB isolates on survival rate, while lowercase notation indicates the effect of incubation time on survival rate. Notation at 3.5 and 6.5% salinity shows no significant difference ( $p > 0.05$ ).

were not significantly different ( $p > 0.05$ ) for 24 h (Table 2). Examples of LAB that are tolerant to bile salt concentrations of 2.5-10% are *L. fermentum* and *L. plantarum* (Balcázar *et al.*, 2008). Bacterial resistance to bile is related to its ability to pass through the small intestinal tract because this part has a relatively high concentration of bile salts and can be toxic to bacteria. Bile salts can activate lipolytic enzymes secreted by the pancreas. These lipolytic enzymes will react with fatty acids on the bacterial cytoplasmic membrane. These bile salts have the ability to dissolve phospholipids, cholesterol and proteins. Bacteria that are resistant to bile salts are bacteria that have the ability to produce bile salt hydrolase (BSH) enzymes. The BSH enzyme changes the toxic nature of bile salts for bacteria to become less toxic by hydrolyzing the bile salts with the BSH enzyme. Several bacteria that belong to the LAB genus are known to produce the BSH enzyme; thus, the physico-chemical properties of bile salts can be changed to become non-toxic for lactic acid bacteria (Liong and Shah, 2005; Chen *et al.*, 2014; Patel *et al.*, 2014).

#### Water salinity tolerance

The results of the LAB tolerance test for water salinity can be seen in Table 3. This study explained that the LAB tolerance to freshwater salinity concentration of 0.5% found a significant difference ( $p < 0.05$ ) between each LAB isolate. The isolates that were significantly different were

KBP 3.3.1 and KBP 3.3, with the survival rate of both isolates being 100%. At 3.5% and 6.5% concentrations, there was no interaction between isolates and incubation time. The survival rates of all isolates at the two concentrations were not significantly different (Table 3). Some of LAB that are tolerant to 6.5% water salinity are *L. plantarum*, *L. mesenteroides*, *E. faecium*, *E. faecalis*, *P. pentosaceus* and *W. confusa* (Thapa *et al.*, 2006).

The population of lactic acid bacteria in the aquatic environment is influenced by the concentration of freshwater salinity (Al Bulushi *et al.*, 2010). Water salinity in each area is different because it is influenced by evaporation, rainfall, river water, sea location and size, ocean currents, wind and humidity (Stanis, 2005; Tubalawony *et al.*, 2012; Slamet, 2019). The LAB obtained from the intestines of mackerel were tested for survival in freshwater salinity (concentrations of 0.5%, 3.5% and 6.5%). This was done because the LAB obtained would be applied to tilapia (Pundir *et al.*, 2013; Ashari *et al.*, 2014). Tilapia usually lives in the range of freshwater salinity concentrations of 0.5-6.5% and 0.4-11% (Hassan *et al.*, 2017; Raj *et al.*, 2019).

#### Auto-aggregation assay

Four isolates, namely, KBP 3.3.1, KBP 3.1, KBP 1.3 and KBP 1.3.1 had a strong auto-aggregation ability (autoaggregation >90%). Moreover, there was no significant difference ( $p > 0.05$ ) between the four isolates

( $p>0.05$ ) (Figure 3). Autoaggregation is one of the requirements in forming biofilms to assist colonization in the intestine and adhere to the intestinal epithelium, thereby preventing adhesion by pathogens (Kos *et al.*, 2003; Aslim *et al.*, 2007). Bacteria can show different autoaggregation abilities, ranging from low (16-35%), moderate (35-50%) and strong ( $>50\%$ ) (Montoro *et al.*, 2016; Fernandez-Pacheco *et al.*, 2018). The ability of autoaggregation depends on the bacterial strain, so it varies greatly among the same bacterial species (Caggia *et al.*, 2015; Das *et al.*, 2016). Good autoaggregation ability is not only seen from the results of *in vitro* tests but is supported by *in vivo* tests because many factors influence these properties, such as the host, defense mechanisms, native microbes in the intestine and peristaltic movements (Caggia *et al.*, 2015).

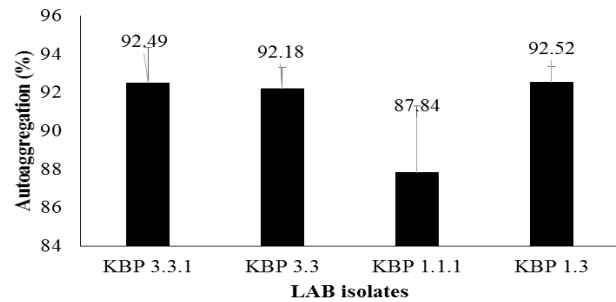
### Enzymatic activity

In Figure 4, there was no interaction between isolates and enzymes produced ( $p>0.05$ ). All of the LAB isolates did not produce amylase enzyme. Isolates of KBP 3.3.1, KBP 3.3, KBP 1.1.1 and KBP 1.3 were not significantly different ( $p>0.05$ ) in producing hydrolase enzymes (Figure 4). Fish need probiotics that have hydrolase enzyme activity to increase the efficiency of feed digestion (Agung *et al.*, 2015). These enzymes include amylase, lipase, protease and cellulase. Hydrolase enzymes play an important role in breaking down starch into disaccharides or trisaccharides and converting peptides into free amino acids (de Souza and Nobre, 2017). Some of LAB that can produce protease enzymes are *W. confusa* KR780676 (Sharma *et al.*, 2018), *W. cibaria* VTT E-153485 and *P. pentosaceus* VTT E-153483 (Rizzello *et al.*, 2018). The probiotics *L. mesenteroides*, *L. plantarum* and *E. faecalis* produce lipase enzymes (Thapa *et al.*, 2006).

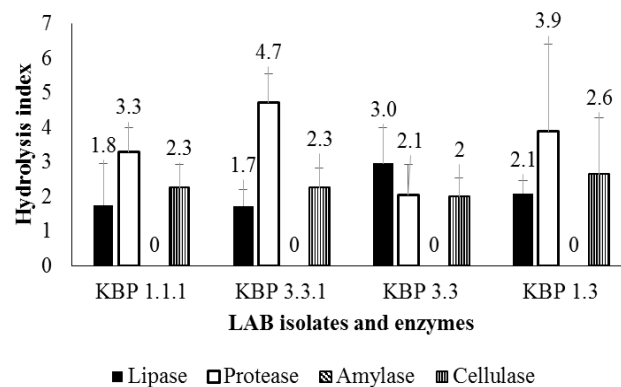
### 16S rDNA based identification of the selected LAB strain

The 16S rDNA analysis suggested that isolates KBP 3.3, KBP 3.3.1 and KBP 1.1.1 were of *W. confusa* 6250 (similarity of 98.8%). The isolate KBP 1.3 was identified as *W. confusa* C5-7 (similarity of 99.8%) (Figure 5). Isolates of KBP 1.1.1, KBP 1.3, KBP 3.3 and 3.3.1 were one species but had different probiotic abilities. The isolate, KBP 1.1.1 showed antimicrobial activity against *S. agalactiae*. However, isolates KBP 3.3 and KBP 1.3 can inhibit *A. hydrophila*, while isolates KBP 3.3.1 can inhibit both pathogens. In addition, the tolerance to environmental conditions such as pH, bile salts, salinity and autoaggregation properties of the four isolates were not significantly different ( $p>0.05$ ). The genus *Weissella* is a native microbe in the gut of tilapia (Del'Duca *et al.*, 2015; Standen *et al.*, 2015; Standen *et al.*, 2016) and mackerel (Zhou *et al.*, 2021).

Not much research is carried out on *W. confusa* isolated from mackerel. However, *W. confusa* has been known as a probiotic for fish. Previous research explained

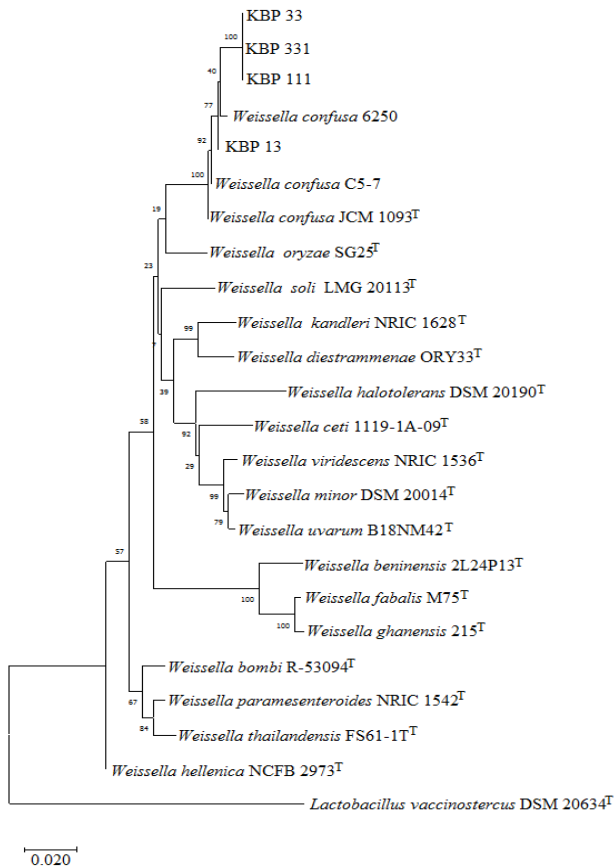


**Figure 3:** The ability of LAB isolates to colonize the intestines of fish. The notation on the graph shows that there is no significant difference ( $p>0.05$ ), and there is no effect of LAB isolates on autoaggregation properties. Data were expressed as the mean from three replicates of each isolate, while the error bars indicate its standard deviation.



**Figure 4:** The activity of lipase, protease, amylase and cellulase enzymes by LAB isolates. Capital notation on the graph shows that there is a significant difference between the types of enzymes produced ( $p<0.05$ ), while lowercase notation shows that there is no significant difference between LAB isolates ( $p>0.05$ ).

that *W. confusa* inhibited the growth of *A. hydrophila* and *S. agalactiae* and added to fish feed significantly supported the growth of seabass (*Lates calcarifer*) ( $p<0.05$ ) (Rengpipat *et al.*, 2008). Moreover, the *W. confusa* LAB-4 also significantly increased the growth (weight and length) of seabass (Rengpipat *et al.*, 2008). Several strains of the *W. confusa* species have been reported as potentially pathogenic (Flaherty *et al.*, 2003; Shin *et al.*, 2007; Lawhon *et al.*, 2014; Fusco *et al.*, 2015). In general, strains of the *W. confusa* are not known to infect healthy humans and animals. However, they are likely to be pathogens that infect humans and animals with immune system disorders. In addition, *W. confusa* has not been officially classified as a potentially pathogenic group by the American Biological Safety Association (ABSA) and the German Committee for Biological Agents. *Weissella confusa* is treated as a Risk group 1 microbe, which means that *W. confusa* does not



**Figure 5:** Phylogenetic tree of probiotic species and the reference strain based on similarity 16S rDNA sequence with Neighbor-Joining algorithm and Tamura-Nei model with a bootstrap of 1000.

cause disease in humans and animals. Therefore, the suggestion for further researchers is that the application of the *W. confusa* strain as a probiotic starter culture must be carried out carefully by selecting strains that do not have pathogenic potential through hemolytic testing (Sturino, 2018).

## CONCLUSION

The LAB isolates, namely KBP 3.3.1, KBP 3.3, KBP 1.1.1 and KBP 1.3, were probiotics for tilapia because they had antimicrobial activity and tolerance to host habitats and produced protease, lipase and cellulase enzymes. The KBP 3.3.1 isolate appears as the best potential candidate as it had the potency to inhibit the two pathogenic bacteria strains, *A. hydrophila* ATCC 7966 and *S. agalactiae* ATCC 13813. Four isolates (KBP 3.3, KBP 1.3 and KBP 1.1.1) were able to inhibit only one of the two strains of the pathogenic bacteria used in the study. Four isolates, namely, KBP 1.1.1, KBP 1.3, KBP 3.3 and KBP 3.3.1 were identified as a *W. confusa*. However, isolates KBP 1.1.1, KBP 3.3 and KBP 3.3.1 were strain 6250, while KBP 1.3 isolate was strain C5-7. The four isolates

were identified with different strains because they had different characteristics and abilities. Further research is required for safety before we use these isolates as probiotics.

## REFERENCES

- Abubakar, S., Subur, R. and Tahir, I. (2019).** Pendugaan ukuran pertama kali matang gonad ikan kembung (*Rastrelliger* sp.) di perairan desa Sidangoli Dehe kecamatan Jailolo Selatan kabupaten Halmahera Barat. *Jurnal Biologi Tropis* **19**(1), 42-51.
- Agung, L. A., Widanarni, and Yuhana, M. (2015).** Application of micro-encapsulated probiotic *Bacillus* NP5 and prebiotic mannan oligosaccharide (MOS) to prevent streptococcosis on tilapia *Oreochromis niloticus*. *Research Journal of Microbiology* **10**(12), 571-581.
- Al Bulushi, I. M., Poole, S. E., Barlow, R., Deeth, H. C. and Dykes, G. A. (2010).** Speciation of gram-positive bacteria in fresh and ambient-stored sub-tropical marine fish. *International Journal of Food Microbiology* **138**(1-2), 32-38.
- Alakomi, H. L., Skyttä, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K. and Helander, I. M. (2001).** Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Application and Environmental Microbiology* **66**, 2001-2005.
- Allameh, S. K., Daud, H., Yusoff, F. M., Saad, C. R. and Ideris, A. (2012).** Isolation, identification and characterization of *Leuconostoc mesenteroides* as a new probiotic from intestine of snakehead fish (*Channa striatus*). *African Journal of Biotechnology* **11**(16), 3810-3816.
- Alonso, S., Castro, M. C., Berdasco, M., de la Banda, I. G., Moreno-Ventas, X. and de Rojas, A. H. (2019).** Isolation and partial characterization of lactic acid bacteria from the gut microbiota of marine fishes for potential application as probiotics in aquaculture. *Probiotics and Antimicrobial Proteins* **11**, 569-579.
- Ashari, C., Tumbol, R. A. and Kolopita, M. E. F. (2014).** Diagnosa penyakit bakterial pada ikan nila (*Oreochromis niloticus*) yang di budi daya pada jaring tancap di danau Tondano. *Budidaya Perairan* **2**(3), 24-30.
- Aslim, B., Onal, D. and Beyatli, Y. (2007).** Factors influencing autoaggregation and aggregation of *Lactobacillus delbrueckii* subsp. *bulgaricus* isolated from handmade yogurt. *Journal of Food Protection* **70**, 223-227.
- Balcázar, J. L., Vendrell D., de Blas, I., Ruiz-Zarzuela, I., Muzquiz, J. L. and Girones, O. (2008).** Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. *Aquaculture* **278**(1-4), 188-191.
- Bestari, N. C. and Suharjono, S. (2015).** Uji kualitatif dan kuantitatif isolat bakteri lipolitik dari limbah cair pabrik pengolahan ikan kecamatan Muncar, Banyuwangi. *Jurnal Biotropika* **3**(3), 151-155.



- Caggia, C., De Angelis, M., Pitino, I., Pino, A. and Randazzo, C. (2015).** Probiotic features of *Lactobacillus* strains isolated from Ragusano and Pecorino Siciliano cheeses. *Food Microbiology* **50**, 109-117.
- Carr, F., Chill, D. and Maida, N. (2002).** The lactic acid bacteria: A literature survey. *Critical Reviews in Microbiology* **28(4)**, 281-370.
- Central Bureau of Statistics of NTT. (2016).** East Nusa Tenggara Province in numbers. CV. Nario Sari, Jakarta.
- Chavasirikunton, V., Vatanyoopaisarn, S. and Phalakornkule, C. (2007).** Bacteriocin-like activity from *Weissella confusa* and *Pediococcus acidilactici* isolated from traditional Thai fermented sausages. *Journal of Culture Collections* **5**, 64-72.
- Chen, C., Chen, X., Jiang, M., Rui, X., Li, W. and Dong, M. (2014).** A newly discovered bacteriocin from *Weissella hellenica* D1501 associated with Chinese Dong fermented meat (Nanx Wudl). *Food Control* **42**, 116-124.
- Chen, S., Liu, C. and Hu, S. (2019).** Dietary administration of probiotic *Paenibacillus ehimensis* NPUST1 with bacteriocin-like activity improves growth performance and immunity against *Aeromonas hydrophila* and *Streptococcus iniae* in Nile tilapia (*Oreochromis niloticus*). *Fish and Shellfish Immunology* **84**, 695-703.
- Collado, M. C., Isolauri, E., Salminen, S. and Sanz, Y. (2009).** The impact of probiotic on gut health. *Current Drug Metabolism* **10**, 68-78.
- Das, P., Khowala, S. and Biswas S. (2016).** *In vitro* probiotic characterization of *Lactobacillus casei* isolated from marine samples. *LWT - Food Science and Technology* **73**, 383-390.
- Dawood, M. A. O., Eweedah, N. M., Moustafa, E. M. and Farahat, E. M. (2019).** Probiotic effects of *Aspergillus oryzae* on the oxidative status, heat shock protein, and immune related gene expression of Nile tilapia (*Oreochromis niloticus*) under hypoxia challenge. *Aquaculture* **520**, 734669.
- Dawood, M. A. O., Zommara, M., Eweedah, N. M., Helal, A. I. and Aboel-Darag, M. A. (2020).** The potential role of nano-selenium and vitamin C on the performances of Nile tilapia (*Oreochromis niloticus*). *Environmental Science and Pollution Research* **27**, 9843-9852.
- de Souza, J. V. and Dias, F. S. (2017).** Protective, technological, and functional properties of select autochthonous lactic acid bacteria from goat dairy products. *Current Opinion in Food Science* **13**, 1-9.
- Del'Duca, A., Cesar, D. E. and Abreu, P. C. (2015).** Bacterial community of pond's water, sediment and in the guts of tilapia (*Oreochromis niloticus*) juveniles characterized by fluorescent *in situ* hybridization technique. *Aquaculture Research* **46(3)**, 707-715.
- Dey, D. K., Koo, B. G., Sharma, C. and Kang, S. C. (2019).** Characterization of *Weissella confusa* DD\_A7 isolated from kimchi. *LWT - Food Science and Technology* **111**, 663-672.
- Dubey, A. K. and Jeevaratnam, K. (2015).** Identification of *Lactobacillus pentosus* and *Weissella confusa* isolated from Uttapam batter fermented with Piper betle leaves. *Malaya Journal of Biosciences* **2**, 13-25.
- Espeche, M. C., Otero, M. C., Sesma, F., Nader-Macias, M. E. F. (2009).** Screening of surface properties and antagonistic substances production by lactic acid bacteria isolated from the mammary gland of healthy and mastitic cows. *Veterinary Microbiology* **135(3-4)**, 346-357.
- Fečkaninová, A., Koščová, J., Mudroňová, D., Schusterová, P., Maruščáková, I. C. and Popelka, P. (2019).** Characterization of two novel lactic acid bacteria isolated from the intestine of rainbow trout (*Oncorhynchus mykiss*, Walbaum) in Slovakia. *Aquaculture* **506**, 294-301.
- Feng, J., Chang, X., Zhang, Y., Yan, X., Zhang, J. and Nie, G. (2019).** Effects of *Lactococcus lactis* from *Cyprinus carpio* L. as probiotics on growth performance, innate immune response and disease resistance against *Aeromonas hydrophila*. *Fish and Shellfish Immunology* **93**, 73-81.
- Feng, J., Wang, L., Zhou, L., Yang, X. and Zhao, X. (2016).** Using *in vitro* immunomodulatory properties of lactic acid bacteria for selection of probiotics against *Salmonella* infection in broiler chicks. *PLoS ONE* **11(1)**, e0147630.
- Fernandez-Pacheco, P., Arévalo-Villena, M., Bevilacqua, A., Corbo, M. R. and Pérez, A. B. (2018).** Probiotic characteristics in *Saccharomyces cerevisiae* strains: Properties for application in food industries. *LWT* **97**, 332-340.
- Flaherty, J. D., Levett, P. N., Dewhirst, F. E., Troe, T. E., Warren, J. R. and Johnson, S. (2003).** Fatal case of endocarditis due to *Weissella confusa*. *Journal of Clinical Microbiology* **41(5)**, 2237-2239.
- Fusco, V., Quero, G. M., Cho, G., Kabisch, J., Meske, D., Neve, H., Bockelmann, W. and Franz, C. M. A. P. (2015).** The genus *Weissella*: Taxonomy, ecology and biotechnological potential. *Frontiers in Microbiology* **6**, 155.
- Habbale, D., Bhargavi, R. and Ramachandra, T. V. (2019).** Saccharification of macroalgal polysaccharides through prioritized cellulase producing bacteria. *Heliyon* **5**: 13-72.
- Hassan, M. A., Noureldin, E. A., Mahmoud, M. A. and Fita, N. A. (2017).** Molecular identification and epizootiology of *Aeromonas veronii* infection among farmed *Oreochromis niloticus* in Eastern Province, KSA. *The Egyptian Journal of Aquatic Research* **43(2)**, 161-167.
- Iwashita, M. K. P., Nakandakare, I. B., Terhune, J. S., Wood, T. and Ranzani-Paiva, M. J. (2015).** Dietary supplementation with *Bacillus subtilis*, *Saccharomyces cerevisiae* and *Aspergillus oryzae* enhance immunity and disease resistance against *Aeromonas hydrophila* and *Streptococcus iniae* infection in juvenile tilapia *Oreochromis niloticus*. *Fish and Shellfish Immunology* **43(1)**, 60-66.

- Jaafar, R. S., Al-Knany, F. N., Mahdi, B. A. and Al-Tae, A. M. R. (2019).** Study the probiotic properties of *Pediococcus pentosaceus* isolated from fish ponds in Basra City, South of Iraq. *Journal of Pure and Applied Microbiology* **13(4)**, 2343-2351.
- Kavitha, M., Raja, M. and Perumal, P. (2018).** Evaluation of probiotic potential of *Bacillus* spp. isolated from the digestive tract of freshwater fish *Labeo calbasu*. *Aquaculture Reports* **11**, 59-69.
- Kong, Y., Li, M., Li, R., Shan, X. and Wang, G. (2020).** Evaluation of cholesterol lowering property and antibacterial activity of two potential lactic acid bacteria isolated from the intestine of snakehead fish (*Channa argus*). *Aquaculture Reports* **17**, 100342.
- Kos, B., Susković, J., Vuković, S., Simpraga, M., Frece, J. and Matosić, S. (2003).** Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *Journal of Applied Microbiology* **94**, 981-987.
- Kuebutornye, F. K. A., Tang, J., Cai, J., Yu, H., Wang, Z., Abarike, E. D., Lu, Y., Li, Y. and Afriyie, G. (2020).** *In vivo* assessment of the probiotic potentials of three host-associated *Bacillus* species on growth performance, health status and disease resistance of *Oreochromis niloticus* against *Streptococcus agalactiae*. *Aquaculture* **527**, 735440.
- Lakra, A. K., Domdi, L., Hanjon, G., Tilwani Y. M. and Arul, V. (2020).** Some probiotic potential of *Weissella confusa* MD1 and *Weissella cibaria* MD2 isolated from fermented batter. *LWT - Food Science and Technology* **125**, 109261.
- Lawhon, S. D., Lopez, F. R., Joswig, A., Black, H. C., Watts, A. E. and Norman, T. E. (2014).** *Weissella confusa* septicemia in a foal. *Journal of Veterinary Diagnostic Investigation* **26**, 150-153.
- Leong, K., Chen, Y., Lin, Y., Pan, S., Yu, B., Wu, H. and Yanagida, F. (2013).** Weissellicin L, a novel bacteriocin from sian-sianzih-isolated *Weissella hellenica* 4-7. *Journal of Applied Microbiology* **115(1)**, 70-76.
- Liong, M. T. and Shah, N. P. (2005).** Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. *Journal of Dairy Science* **88(1)**, 55-66.
- Maragkoudakis, P. A., Zoumpoulou, G., Miaris, C., Kalantzopoulos, G., Pot, B. and Tsakalidou, E. (2006).** Probiotic potential of *Lactobacillus* strains isolated from dairy products. *International Dairy Journal* **16(3)**, 189-199.
- Masuda, Y., Zendo, T., Sawa, N., Perez, R. H., Nakayama, J. and Sonomoto, K. (2011).** Characterization and identification of weissellicin Y and weissellicin M, novel bacteriocins produced by *Weissella hellenica* QU 13. *Journal of Applied Microbiology* **112(1)**, 99-108.
- Mohammadi, G., Rafiee, G., Basuini, M. F. E., Abdel-Latif, H. M. R. and Dawood, M. A. O. (2020).** The growth performance, antioxidant capacity, immunological responses, and the resistance against *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*) fed *Pistacia vera* hulls derived polysaccharide. *Fish and Shellfish Immunology* **106**, 36-43.
- Montoro, B. P., Benomar, N., Lerma, L. L., Gutiérrez, S. C., Gálvez, A. and Abriouel, H. (2016).** Fermented Aloreña table olives as a source of potential probiotic *Lactobacillus pentosus* strains. *Frontiers in Microbiology* **7**, 1583.
- Mouriño, J. L. P., Pereira, G. V., Vieira, F. N., Jatobá, A. B., Ushizima, T. T., da Silva, B. C., Seiffert, W. Q., Jesus, G. F. A. and Martins, M. L. (2016).** Isolation of probiotic bacteria from the hybrid South American catfish *Pseudoplatystoma reticulatum* × *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae): A haematological approach. *Aquaculture Reports* **3**, 166-171.
- Muñoz-Atienza, E., Gómez-Sala, B., Araújo, C., Campanero, C., del Campo, R., Hernández, P. E., Herranz, C. and Cintas, L. M. (2013).** Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. *BMC Microbiology* **13**, 15.
- Ng, S. Y., Koon, S. S., Padam, B. S. and Chye, F. Y. (2015).** Evaluation of probiotic potential of lactic acid bacteria from traditional Malaysian fermented *Bambangan* (*Mangifera pajang*). *Journal of Food* **13(4)**, 563-572.
- Padmavathi, T., Bhargavi, R., Priyanka, P. R., Niranjan, N. R. and Pavitra, P. V. (2018).** Screening of potential probiotic lactic acid bacteria and production of amylase and its partial purification. *Journal of Genetic Engineering and Biotechnology* **16(2)**, 357-362.
- Pal, A. and Ramana, K. V. (2010).** Purification and characterization of bacteriocin from *Weissella paramesenteriodes* DFR-8, an isolate from cucumber (*Cucumis sativus*). *Journal of Food Biochemistry* **34(5)**, 932-948.
- Patel, A., Prajapati, J. B., Holst, O. and Ljungh, A. (2014).** Determining probiotic potential of exopolysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented food products. *Food Bioscience* **5**, 27-33.
- Pinpimai, K., Rodkhum, C., Chansue, N., Katagiri, T., Maita, M. and Pirarat, N. (2015).** The study on the candidate probiotic properties of encapsulated yeast, *Saccharomyces cerevisiae* JCM 7255, in Nile Tilapia (*Oreochromis niloticus*). *Research in Veterinary Science* **102**, 103-111.
- Prahadina, V. D., Boer, M. and Fahrudin, A. (2015).** Sumberdaya ikan kembung (*Rastrelliger kanagurta* Cuvier 1817) di perairan selat Sunda yang didaratkan di PPP Labuan, Banten. *Marine Fisheries* **6(2)**, 169-175.
- Pundir, R. K., Rana, S., Kashyap, N. and Kaur, A. (2013).** Probiotic potential of lactic acid bacteria isolated from food samples: An *in vitro* study. *Journal of Applied Pharmaceutical Science* **3(3)**, 85-93.
- Raj, N. S., Swaminathan, T. R., Dharmaratnam, A., Raja, S. A., Ramraj, D. and Lal, K. K. (2019).**

- Aeromonas veronii* caused bilateral exophthalmia and mass mortality in cultured Nile tilapia, *Oreochromis niloticus* (L.) in India. *Aquaculture* **512**, 734278.
- Rajoka, M. Sh. R., Hayat, H. F., Sarwar, S., Mehwish, H. M., Ahmad, F., Hussain, N., Shah, S. Z. H., Khurshid, M., Siddiqui, M. and Shi, J. (2018).** Isolation and evaluation of probiotic potential of lactic acid bacteria isolated from poultry intestine. *Microbiology* **87**, 116-126.
- Ramesh, D., Vinothkanna, A., Rai, A. K. and Vignesh, V. S. (2015).** Isolation of potential probiotic *Bacillus* spp. and assessment of their subcellular components to induce immune responses in *Labeo rohita* against *Aeromonas hydrophila*. *Fish and Shellfish Immunology* **45(2)**, 268-276.
- Rengpipat, S., Rueangruklikhit, T. and Piyatiratitivorakul, S. (2008).** Evaluations of lactic acid bacteria as probiotics for juvenile seabass *Lates calcarifer*. *Aquaculture Research* **39(2)**, 134-143.
- Rizzello, C. G., Coda, R., Wang, Y., Verni, M., Kajala, I., Katina, K. and Laitila, A. (2018).** Characterization of indigenous *Pediococcus pentosaceus*, *Leuconostoc kimchii*, *Weissella cibaria* and *Weissella confusa* for faba bean bioprocessing. *International Journal of Food Microbiology* **302**, 24-34.
- Shahid, M., Hussain, B., Riaz, D., Khurshid, M., Ismail, M. and Tariq, M. (2017).** Identification and partial characterization of potential probiotic lactic acid bacteria in freshwater *Labeo rohita* and *Cirrhinus mrigala*. *Aquaculture Research* **48(4)**, 1688-1698.
- Sharma, S., Kandasamy, S., Kavitate, D. and Shetty, P. H. (2018).** Probiotic characterization and antioxidant properties of *Weissella confusa* KR780676, isolated from an Indian fermented food. *LWT - Food Science and Technology* **97**, 53-60.
- Shin, J. H., Kim, D. I., Kim, H. R., Kim, D. S., Kook, J. K. and Lee, J. N. (2007).** Severe infective endocarditis of native valves caused by *Weissella confusa* detected incidentally on electrocardiography. *Journal of Infection* **54(3)**, 149-151.
- Slamet, A. (2019).** Analisis massa air di Perairan Utara Pulau Lembata, Nusa Tenggara Timur. BSc. Thesis. Bogor Agricultural University, Bogor.
- Srionnual, S., Yanagida, F., Lin, L., Hsiao, K. and Chen, Y. (2007).** Weissellicin 110, a newly discovered bacteriocin from *Weissella cibaria* 110, isolated from Plaa-Som, a fermented fish product from Thailand. *Applied and Environmental Microbiology* **73(7)**, 2247-2250.
- Standen, B. T., Peggs, D. L., Rawling, M. D., Foey, A., Davies, S. J., Santos, G. A. and Merrifield, D. L. (2016).** Dietary administration of a commercial mixed-species probiotic improves growth performance and modulates the intestinal immunity of tilapia, *Oreochromis niloticus*. *Fish and Shellfish Immunology* **49**, 427-435.
- Standen, B. T., Rodiles, A., Peggs, D. L., Davies, S. J., Santos, G. A. and Merrifield, D. L. (2015).** Modulation of the intestinal microbiota and morphology of tilapia, *Oreochromis niloticus*, following the application of a multi-species probiotic. *Applied Microbiology and Biotechnology* **99**, 8403-8417.
- Stanis, S. (2005).** Pengelolaan sumberdaya pesisir dan laut melalui pemberdayaan kearifan lokal di kabupaten Lembata Propinsi Nusa Tenggara Timur. Thesis. Pascasarjana Universitas Diponegoro, Semarang.
- Sturino, J. M. (2018).** Literature-based safety assessment of an agriculture- and animal-associated microorganism: *Weissella confusa*. *Regulatory Toxicology and Pharmacology* **95**, 142-152.
- Suárez, J. E. (2015).** Microbiota autóctona, probióticos y prebióticos. *Nutricion Hospitalaria* **31**, 3-9.
- Tan, H. Y., Chen, S. and Hu, S. (2019).** Improvements in the growth performance, immunity, disease resistance, and gut microbiota by the probiotic *Rummeliibacillus stabekisii* in Nile tilapia (*Oreochromis niloticus*). *Fish and Shellfish Immunology* **92**, 265-275.
- Thapa, N., Pal, J. and Tamang, J. P. (2006).** Phenotypic identification and technological properties of lactic acid bacteria isolated from traditionally processed fish products of the Eastern Himalayas. *International Journal of Food Microbiology* **107**, 33-38.
- Tubalawony, S., Kusmanto, E. and Muhadjirin. (2012).** Suhu dan salinitas permukaan merupakan indikator *upwelling* sebagai respon terhadap angin muson tenggara di perairan bagian utara Laut Sawu. *Ilmu Kelautan* **17(4)**, 226-239.
- Vijayakumar, M., Ilavenil, S., Kim, D. H., Arasu, M. V., Priya, K. and Choi, K. C. (2015).** *In-vitro* assessment of the probiotic potential of *Lactobacillus plantarum* KCC-24 isolated from Italian rye-grass (*Lolium multiflorum*) forage. *Anaerobe* **32**, 90-97.
- Wang, W., Liu, W. and Chu, W. (2020).** Isolation and preliminary screening of potentially probiotic *Weissella confusa* strains from healthy human feces by culturomics. *Microbial Pathogenesis* **147**, 104356.
- Woraprayote, W., Pumpuang, L., Tosukhowong, A., Roytrakul, S., Perez, R. H., Zendo, T., Sonomoto, K., Benjakul, S. and Visessanguan, W. (2015).** Two putatively novel bacteriocins active against Gram-negative food borne pathogens produced by *Weissella hellenica* BCC 7293. *Food Control* **55**, 176-184.
- Wulandari, E., Yurmiati, H., Subroto, T. and Suradi, K. (2020).** Quality and probiotic lactic acid bacteria diversity of rabbit meat *Bekasam*-fermented meat. *Food Science of Animal Resources* **40(3)**, 362-376.
- Yilmaz, S. (2019).** Effects of dietary caffeic acid supplement on antioxidant, immunological and liver gene expression responses, and resistance of Nile tilapia, *Oreochromis niloticus* to *Aeromonas veronii*. *Fish and Shellfish Immunology* **86**, 384-392.
- Zhou, Y., Wu, S., Peng, Y., Jin, Y., Xu, D. and Xu, X. (2021).** Effect of lactic acid bacteria on mackerel (*Pneumatophorus japonicus*) seasoning quality and flavor during fermentation. *Food Bioscience* **41**, 100971.
- Zidni, I., Afrianto, E., Mahdiana, I. and Herawati, H. (2017).** Laju pengosongan lambung ikan mas

(*Cyprinus carpio*) dan ikan nila (*Oreochromis niloticus*). *Jurnal Perikanan dan Kelautan* **9(2)**, 147-151.