J. Pijar MIPA, Vol. 18 No. 5, September 2023: 788-794 ISSN 1907-1744 (Print)
DOI: 10.29303/jpm.v18i5.5481 ISSN 2460-1500 (Online)

# ISOLATION AND IDENTIFICATION OF PROTEOLYTIC LACTIC ACID BACTERIA FROM FRESHWATER POMFRET (*Colossoma macropomum*) INTESTINE

**Risti Wulaningtyas* and Rudiana Agustini**
Chemistry Department, Faculty of Mathematics and Natural Sciences, Univesitas Negeri Surabaya, Surabaya, Indonesia
*Email: wulanristi75@gmail.com

Received: August 2, 2023. Accepted: August 18, 2023. Published: September 30, 2023

**Abstract:** The production of freshwater pomfret (*Colossoma macropomum*) in Indonesia is abundant, but the digestive tract waste weighing 10-15% of the fish body is not widely utilized. The fish's digestive tract can potentially produce lactic acid bacteria (LAB). Lactic acid bacteria have a proteolytic system that can produce extracellular protease enzymes. Protease enzymes are the most widely utilized enzymes in various industries and have a global revenue of up to $2.1 billion in 2021. Protease enzymes derived from microbes are in great demand due to the efficiency of time, place, cost, and better cultivation techniques than animal and plant sources. This study aimed to isolate and identify proteolytic lactic acid bacteria from the intestines of freshwater pomfret fish and determine their proteolytic index. The methods used were morphological, biochemical, sugar, and proteolytic tests. The BAT-A isolate with the highest proteolytic index of 2.52 with characteristics of Gram-positive, non-spore, facultatively anaerobic, catalase-negative, non-motile, homofermentative, can ferment glucose, maltose, lactose and sucrose which were then identified as proteolytic lactic acid bacteria *Enterococcus sp* which has the potential as a candidate source of protease enzymes from bacteria isolated from the intestines of freshwater pomfret.

**Keywords:** *LAB, Enterococcus, Freshwater Pomfret, Proteolytic, Intestine*

## INTRODUCTION

Freshwater pomfret (*Colossoma macropomum*) has great potential to be utilized. The production of freshwater pomfret fish seeds in Indonesia increases every year; the data in 2014-2016 were 550,210,00 heads to 1,453,810,000 heads [1]. The utilization of freshwater pomfret commodities is generally only consumed by the public as fillet meat, which is only about 38% of the fish body; the rest is waste in the form of bones, scales, and fish entrails. Some fish species are reported to have viscera weighing up to 10-15% of their body weight [2].

The market revenue of protease enzymes globally is estimated to reach $2.21 billion by 2021 and is the most dominant enzyme utilization [3]. Protease production from animal sources cannot meet the worldwide industrial demand. Therefore, microbial sources of protease enzymes are the most preferred alternative for commercial production. This is due to various advantages, including rapid microbial growth, cultivation methods, and relatively easy genetic manipulation protocols [3], [4].

Several studies have found various species of proteolytic lactic acid bacteria derived from the digestive tract of animals, especially fish [5]. The research of Govindaraj et al. (2021) isolated LAB from the intestines of freshwater fish, which showed significant hydrophobicity and secretion of extracellular enzymes such as amylase, protease, and lipase[6].

Efforts to isolate and identify the target proteolytic lactic acid bacteria from the intestines of freshwater pomfret need to be carried out to obtain a candidate source of protease enzymes that are effective as an initiation of the utilization of fish guts, especially the intestinal organs of freshwater pomfret, have not been widely carried out. This study aims to isolate and identify proteolytic lactic acid bacteria isolates found in the intestines of freshwater pomfret (Colossoma macropomum) and determine the index value of their proteolytic activity.

## RESEARCH METHODS

The materials used in this study included 10 freshwater pomfret fish, Merck MRS agar selective media, Merck MRS broth, Himedia Skim Milk Agar, Phenol Red media, glucose, Himedia SIM media, Merck $CaCO_3$, OneMed 70% alcohol, OneMed 96% alcohol, Lugol, Otsu 0.9% physiological NaCl solution, distilled water, crystal violet, safranin, malachite green and OneMed 3% $H_2O_2$.

Tools used include tweezers, knife, petri dish, test tube, Hirayama autoclave, Mermet incubator, glass slide, Eppendorf micropipette, Nikon light microscope, magnetic stirrer, vortex and Laminar Air Flow.

### Media preparation

200 ml of physiological NaCl was added to an Erlenmeyer flask along with 13.4 grams of MRS agar media and one gram of $CaCO_3$, stirred until homogeneous. 15 ml of MRS agar and $CaCO_3$ media mixture was poured into petri dishes after autoclaving for 15 minutes at 121°C and 1 atm pressure [5].

### Bacterial isolation

Proteolytic lactic acid bacteria were isolated by planting (inoculation) of bacteria starting with a

multistage dilution process. One gram of mashed Pomfret gut was diluted into 9 ml of physiological NaCl in a test tube ($10^{-1}$ series). Dilution continued until the $10^{-8}$ series, and then injection was carried out by taking 1 ml of sample using a micropipette from dilutions $10^{-4}$ to $10^{-8}$ poured into each Petri dish containing solid MRS agar media. Petri dishes are then shaken, like in Figure 8, until the sample is evenly distributed, then at 37ºC for 24 hours, the incubation process is carried out [5].

**Purification of isolates**

Select five colonies with the largest clear zone, perfectly round colonies, flat colony edges, milky white or yellowish color, and convex colony elevation. The selected colonies were purified using the four quadrant scratch technique aseptically onto MRS agar solid media; then, incubation was carried out at 37ºC for 24 hours. The purification stage with the scratch method was repeated 5-7 times to obtain truly pure colonies [7],[8].

**Morphological test**

Morphological tests include observation of isolate shape, Gram staining, and spore staining. Gram staining is done with crystal violet as a primary dye, lugol, distilled water, 96% alcohol, and safranin solution as a secondary dye. Gram-positive bacteria are characterized by purple bacterial cells, and Gram-negative bacteria are characterized by red bacterial cells [9].

Spore staining is done by the Schaeffer-Fulton method through heating using 1% malachite green solution as the primary color, distilled water, and safranin solution as the secondary color. Spore-forming bacteria are colored green-blue, and non-sporing bacteria are colored red. Observations were made under a light microscope at 400x and 1000x magnification [10].

**Biochemical test**

1. Oxygen response test

One or two doses of pure bacterial isolates were inoculated into liquid MRS broth media homogenized with a vortex to determine the type of respiration. For 24 hours, the incubation process was carried out at 37°C. Observations focused on the location of bacterial growth [11].

2. Catalase test

A sterile glass slide was applied with one or two ounces of pure bacterial isolate and then an excess of 3% $H_2O_2$ solution with a dropper pipette. We observed gas bubbles formed on the object glass. The presence of gas bubbles that appear is a sign of the presence of catalase enzyme produced by bacteria[12].

3. Motility test

The autoclaved SIM media was added to a test tube with a lid of 10 ml and waited until it was semi-solid; an *Ose* needle containing bacterial isolates up to a few centimeters for 24 hours; the incubation process was carried out at 37°C. The results show positive when bacterial growth is spread throughout the media, observed based on the color of the media, which becomes cloudy. Non-motile results (negative) if bacterial growth does not spread and visible traces of *Ose* needle puncture [13].

4. Sugar test

Media for the sugar test is phenol red broth base media to which various sugars are added at a concentration of 0.1% (w/v). A Durham tube was placed in a test tube to observe gas production, and 5 ml of media was added. One *Ose* of pure bacteria was aseptically inoculated into the media and incubated for 24 hours at 37°C. A yellow color change indicates success and bubbles trapped in the Durham tube indicate gas production [14].

5. Proteolytic test

Bacterial inoculation is carried out by bottling bacterial isolates with an *Ose* needle into skim milk media that has hardened as many as 5 points, followed by incubation at 37°C within 24-48 hours [5]. A clear zone around the colony indicates the presence of proteolytic activity in bacteria. The proteolytic index value was obtained based on the formula:

$$PI = \frac{\text{Clear zone diameter - colony diameter}}{\text{colony diameter}}$$

**Data analysis**

The descriptive data analysis method was used to analyze the research data. Data collection based on morphological, biochemical, and sugar tests was used to identify bacterial species based on the book Determinative Bacteriology by Bergey (1994) and protease enzyme activity tests based on proteolytic index.

## RESULTS AND DISCUSSION

**Isolation and purification of bacteria**

Bacterial cultivation of dilution series $10^{-4}$ to $10^{-8}$ by pour plate method on MRS agar media resulted in the number of colonies more than 300 colonies on cups $10^{-4}$ and $10^{-5}$, 239 bacterial colonies on petri dish$10^{-6}$, 49 colonies on petri dish$10^{-7}$ and 1 colony on petri dish$10^{-8}$. The colonies were selected for purification from cups $10^{-6}$ and $10^{-7}$ so that they were easy to observe and the distance between colonies was separate. Two colonies were taken from plates $10^{-6}$ and three from plates $10^{-7}$, producing clear zones, perfectly round, milky white, flat colony edges, and convex colony elevations. The five isolates were purified 7 times with the four-quadrant streak method. Then, the five isolates were named BAT-A, BAT-B, BAT-C, BAT-D, and BAT-E.

**Morphological test**

1. Gram staining

The Gram staining results showed that the five isolates were gram-positive; the cells were purple after being observed on a light microscope at 400x magnification. Cell shape was also observed under a light microscope at 1000x magnification at the Gram observation stage. Five target isolates have a locus cell shape (round), as shown in Figure 1.

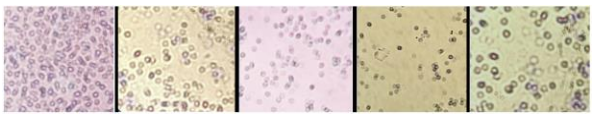

Figure 1. Gram staining of BAT-A, BAT-B, BAT-C, BAT-D and BAT-E isolates

2. Spore staining

Observation of spores carried out under a light microscope at 400x magnification resulted in the five isolates having no spores or non-spores that cannot maintain the green color of malachite green, seen that the cells are red from the safranin solution, which can be observed in Figure 2.

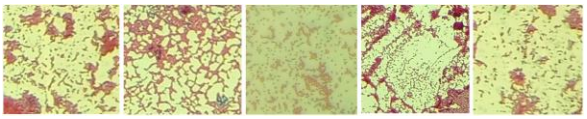

Figure 2. Spore staining of BAT-A, BAT-B, BAT-C, BAT-D and BAT-E isolates

**Biochemical test**

1. Oxygen response test

The data obtained after the bacteria were inoculated on MRS broth media shows that the five isolates have a type of facultative anaerobic respiration, which is the ability of bacteria to live in the presence of an oxygen supply but can also survive without an oxygen supply. Bacterial growth collected at the bottom of the tube and partially floating in the middle indicates bacterial growth away from the oxygen source, namely the upper surface, shown in Figure 3.

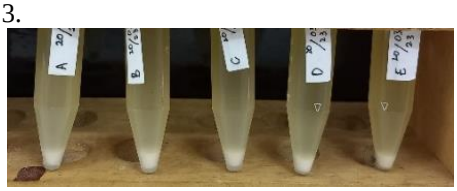

Figure 3. Oxygen response of BAT-A, BAT-B, BAT-C, BAT-D and BAT-E isolates

2. Catalase test

Based on the data, the five isolates show negative catalase results when dripped with 3% H2O2, which does not produce oxygen bubbles, meaning that the bacteria do not produce catalase enzymes that react with H2O2.

Motility test

The data obtained from the motility test, namely five isolates of lactic acid bacteria from the intestines of freshwater pomfret fish, showed negative or non-motile results. It can be seen in Figure 4 that the puncture marks on the semi-solid SIM media are straight lines without spreading, and the color of the media is not cloudy due to the absence of bacterial growth that spreads.

Figure 4. Motility test of BAT-A, BAT-B, BAT-C, BAT-D and BAT-E isolate

Table 1. Morphological and biochemical test results

| Test | BAT-A | BAT-B | BAT-C | BAT-D | BAT-E |
|---|---|---|---|---|---|
| Colony Shape | Round | Round | Round | Round | Round |
| Color | Milky White | Milky White | Milky White | Milky White | Milky White |
| Margin | Regular | Regular | Regular | Regular | Regular |
| Elevation | Convex | Convex | Convex | Convex | Convex |
| Cell Shape | Coccus | Coccus | Coccus | Coccus | Coccus |
| Gram | + | + | + | + | + |
| Spore | - | - | - | - | - |
| Response $O_2$ | FA | FA | FA | FA | FA |
| Catalase | - | - | - | - | - |
| Motility | - | - | - | - | - |

Note: FA = Facultative Anaerob; + = positive; - = negative

3. Proteolytic test

Bacterial isolates with characteristics of lactic acid bacteria, namely gram-positive, non-spore, catalase-negative, and non-motile, are then subjected to proteolytic tests to determine their potential to produce protease enzymes. Five isolates of lactic acid bacteria inoculated on skim milk agar media produce a clear zone, thus indicating that the five isolates have proteolytic activity. The proteolytic index calculated from 5 inoculation points is presented in Table 2 as follows. Based on the data, isolate BAT-A has the highest proteolytic index of 2.52, and isolate BAT-C has the lowest proteolytic index of 1.29.

Table 2. Proteolytic test results

| Isolate | PI 1 | PI 2 | PI 3 | PI 4 | PI 5 | Average |
|---|---|---|---|---|---|---|
| BAT-A | 3.5 | 2.6 | 1.67 | 2.13 | 2.71 | 2.52 |
| BAT-B | 2.6 | 2.8 | 2.63 | 2 | 1.67 | 2.34 |
| BAT-C | 1.5 | 2 | 1.17 | 0.78 | 1 | 1.29 |
| BAT-D | 1.57 | 2.8 | 2.5 | 1.3 | 1.1 | 1.85 |
| BAT-E | 1 | 1 | 1.9 | 0.85 | 2.17 | 1.38 |

Note: PI = Proteolytic Index

Sugar test

The sugar test was conducted on BAT-A isolate, which has the highest proteolytic index. Based

on the data, BAT-A isolate can ferment glucose, maltose, lactose, and sucrose. In this test, the type of fermentation is also known, namely BAT-A isolate is homofermentative bacteria, which means that it only produces one main product in its fermentation, namely lactic acid, which is known from the change in media color from reddish to yellow due to the acid formed. In this test, it is also known that BAT-A isolate cannot ferment sorbitol and mannitol. Based on morphological test data, biochemical tests, and sugar tests, the identification of bacteria based on Bergey's Determinative Bacteriology 9th Edition book is known that BAT-A isolates are lactic acid bacteria of the Enterococcus genus that have proteolytic activity.

## Discussion

### Isolation and purification of bacteria

Isolation separates one type of bacteria from its original environment by inoculating a small sample into culture media. The use of 0.9% physiological NaCl in the dilution series to maintain the same osmotic pressure as the usual environment of living bacteria prevents the rupture of bacterial cells due to the difference in osmotic pressure inside the cell compared to outside the bacterial cell. Two colonies were taken from petri dish10-6 and three from petri dish10-7, producing clear zones, perfectly round, milky white, flat colony edges, and convex colony elevations. Isolated lactic acid bacteria that have round-shaped colony morphology, milky white, flat edges, and convex elevations [7,15].

Purification of 5 isolates was carried out with the four-quadrant method 7 times. The principle of isolate purification is to separate 1 type of bacteria from a mixed culture by inoculating 1 separate colony into a new medium. Purifying bacteria is important in the identification process so there are no test errors due to contaminants or different types of bacteria in 1 isolate culture [8]. The pure isolates obtained were BAT-A, BAT-B, BAT-C, BAT-D and BAT-E. Pure isolates were inoculated on MRS broth as stock culture.

### Morphology test

Testing and observing the morphology of bacteria is an important part of studying the nature of an organism. Physical characteristics affect biological functions, including how to adapt and obtain nutrients to interact with other organisms, not just to see the physical form of an organism [16].

### Gram staining

Gram staining aims to determine the nature of a bacterium with gram-positive or gram-negative characteristics with differential staining techniques using crystal violet solution as a primary dye and safranin solution as a secondary dye. The nature of Gram is based on the structure of the cell wall owned by bacteria. Gram-positive bacteria have a thicker cell wall morphological structure and are dominated by peptidoglycan (around 60-90%) compared to Gram-negative bacteria. The thick layer of peptidoglycan in Gram-positive bacteria will be hydrated by alcohol, causing the pores of the bacterial cell wall to close and block the exit of the violet-iodine crystal complex from the cell [9,17].

Cell shape was also observed under a light microscope at 1000x magnification at the Gram color observation stage. Five isolates, BAT-A, BAT-B, BAT-C, BAT-D, and BAT-E, have a cocci (round) cell shape. The nature of Gram-positive bacteria and round-shaped cells is also found in research on lactic acid bacteria isolates from the digestive tract of fish such as eel fish [18] and milkfish [15]. Bacteria with Gram-positive and cocci-shaped properties are also common morphological characteristics of lactic acid bacteria.

### Spore staining

Spore staining aims to determine the presence or absence of spore structures in bacteria with the Schaeffer-Fulton method using malachite green and safranin dyes. Non-spore lactic acid bacteria cells cannot retain the green color of malachite green. The existence of spore structures in bacteria is related to how to defend themselves in extreme environments such as temperature, humidity, and harmful chemicals. Bacteria with spore cells can bind and maintain the dye so that flushing with distilled water has no effect because the spores have a hard and thick sheath structure. In nonsporic bacteria, after rinsing with distilled water and then stained with safranin, the cells are red as a marker of bacteria that do not have a spore structure based on the Scheffer-Fulton method. The findings follow the research of Irwansyah (2018) in his research on lactic acid bacteria from the digestive tract of star pomfret fish with characteristics that do not have a spore structure [19].

### Biochemical test

1. Oxygen response test

Anaerobic, facultative bacteria respiration type is a unique group of bacteria that can grow and develop with or without oxygen. From an evolutionary perspective and concerning oxygen demand, facultative anaerobic bacteria have the microenvironment's most widespread and diffuse capacity [20].

2. Catalase test

The catalase test is performed to determine the biological properties of bacteria in secreting the enzyme catalase. Catalase is an enzyme produced by some species of bacteria. This enzyme protects bacteria from hydrogen peroxide (H2O2), which can damage and kill them. Catalase will convert hydrogen peroxide into water molecules (H2O) and oxygen gas (O2). As a result, if there is abundant hydrogen peroxide, it will make catalase very active so that

there is a rapid increase in O2 production, resulting in bubbles [12]. One of the common characteristics of lactic acid bacteria is that these bacteria do not secrete the enzyme catalase [21].

3. Motility test

A motility test determines the movement or distribution of bacteria on the growth media. This motility test is based on the presence or absence of flagella structures in a bacterium. Motility is defined as the movement of cells in some form of movement. Many bacterial cells are motile because it allows them to escape from unfavorable conditions and exploit new resources or environments. Combined with chemotaxis, the ability to sense chemical compounds and other movements, bacteria can take nutrients and reach specific niches. In this sense, motility is also included in the interaction between microorganisms and their hosts[22]. Non-motile properties in lactic acid bacteria were also found in Irwansyah's (2018) research on lactic acid bacteria isolates from the digestive tract of star Pomfret [19].

4. Proteolytic test

The five isolates of the target lactic acid bacteria produced clear zones on skim milk agar media, thus indicating that the five isolates had proteolytic activity. Bacteria that produce clear zones on skim milk agar media indicate their ability to produce extracellular protease enzymes. Extracellular proteases are important for the consumption and hydrolysis of proteinaceous nutrients. Extracellular proteases have significant roles and several applications in different industries. Microbially derived proteases account for about 40% of all global enzyme sales [23]. The results are in line with research on isolating proteolytic bacteria from the catfish digestive tract [24], eel [18], and Bonylip barb [25], which produced isolates of lactic acid bacteria with proteolytic activity.

Proteolytic activity exists because there is a proteolytic system in lactic acid bacteria. In the process, this system can produce peptides and amino acids through the hydrolysis of protein compounds into simpler forms of compounds. Lactic acid bacteria cannot synthesize amino acids for their metabolic needs, so they need proteins from outside to degrade and utilize the amino acids produced [26].

The ability of bacteria to produce protease enzymes can now be further developed into protease enzyme producers for industry. As part of environmentally friendly technology, the application of protease is very broad, having promising potential for the future. The last few decades have seen the exploration of the application of this enzyme in other industries, not only food technology but also textile, leather, detergent, agriculture, and pharmaceutical industries [26].

5. Sugar test

This sugar test determines the characteristics of bacteria, namely their ability to ferment various types of carbohydrates and determine the type of fermentation. Lactic acid bacteria can ferment carbohydrate types because carbohydrates are the main carbon source of energy [27-28].

Based on morphological test data, biochemical tests, and sugar tests, the characteristics of BAT-A isolates, according to Bergey's Determination Bacteriology, are included in the *Enterococcus* genus. *Enterococcus* is a microorganism in various places, including water, plants, soil, food, and the digestive tract of animals and humans. *Enterococcus* has been widely used as a starter in food fermentation because of its biotechnological properties, namely enzymatic activity, especially proteolytic [29].

In previous studies, there were also several findings of Enterococcus genus lactic acid bacteria in the digestive tract of fish and processed fish products that have proteolytic activity, such as the isolation of lactic acid bacteria from anchovies found *E faecium, E faecalis*, and *E durans* [30], glass fish intestines found Enterococcus sp [31] and Tor fish digestive tract found E. faecalis [32].

## CONCLUSION

Proteolytic lactic acid bacteria isolates obtained from intestinal samples of freshwater pomfret (Colossoma macropomum) are 5 isolates with similar characteristics. Isolate BAT-A has the highest proteolytic index of 2.52 and can ferment carbohydrate types of glucose, lactose, maltose, and fructose with homofermentative fermentation type, so based on its characteristics, it is identified as Enterococcus sp. Enterococcus sp bacterial isolates from freshwater pomfret intestines can be an alternative source of protease enzymes the industry needs.

## REFERENCES

[1] Pusat Data Statistik dan Informasi. (2018). *Kelautan dan Perikanan dalam Angka Tahun 2018*. Pusat Data, Statistik dan Informasi.

[2] Purwaningrum, S. D., & Sukaryo, S. (2018). Uji Karakteristik Biodiesel Berbahan Dasar Limbah Jeroan Ikan Diproses Menggunakan Mikrogelombang. *METANA*.

[3] Mokashe, N., Chaudhari, B., & Patil, U. (2018). Operative Utility Of Salt-Stable Proteases Of Halophilic And Halotolerant Bacteria In The Biotechnology Sector. *International Journal of Biological Macromolecules*, *117*(2017), 493–522. https://doi.org/10.1016/j.ijbiomac.2018.05.217

[4] Razzaq, A., Shamsi, S., Ali, A., Ali, Q., Sajjad, M., Malik, A., & Ashraf, M. (2019). Microbial proteases applications. *Frontiers in Bioengineering and Biotechnology*, *7*(JUN), 1–20.

[5] Tjahjaningsih, W., Masithah, E. D., Pramono, H., & Suciati, P. (2016). Aktivitas Enzimatis Isolat Bakteri Asam Laktat dari Saluran Pencernaan Kepiting Bakau (*Scylla spp*.) Sebagai Kandidat Probiotik. *Jurnal Ilmiah Perikanan Dan Kelautan*, *8*(2), 94–108.

[6] Govindaraj, K., Samayanpaulraj, V., Narayanadoss, V., & Uthandakalaipandian, R. (2021). Isolation of Lactic Acid Bacteria from Intestine of Freshwater Fishes and Elucidation of Probiotic Potential for Aquaculture Application. *Probiotics and Antimicrobial Proteins*, *13*(6), 1598–1610.

[7] Finanda, A., Mukarlina, & Rahmawati. (2021). Isolasi Dan Karakterisasi Genus Bakteri Asam Laktat Dari Fermentasi Daging Buah. *Protobiont*, *10*(2), 37–41

[8] Jufri, R. F. (2020). Microbial Isolation. *Journal La Lifesci*, *1*(1), 18–23.

[9] O'Toole, G. A. (2016). Classic spotlight: How the gram stain works. *Journal of Bacteriology*, *198*(23), 3128–3128.

[10] Fitrah, I. D., -, D., & -, R. (2013). Isolasi Pasteurella Multocida Pada Kuda Dan Sensitivitasnya Terhadap Antibiotik. *Jurnal Medika Veterinaria*, *7*(2), 121–125.

[11] Sari, R., & Prayudyaningsih, R. (2017). Karakter Isolat Rhizobia Dari Tanah Bekas Tambang Nikel Dalam Memanfaatkan Oksigen Untuk Proses Metabolismenya. *Info Teknis EBONI*, *14*(2), 123–136.

[12] Al-Joda, B. M. S., & Jasim, A. H. (2021). Biochemical Testing Revision For Identification Several Kinds of Bacteria. *Journal of University of Babylon*, *29*(2), 168–176. www.journalofbabylon.com

[13] Ulfa, A., Suarsini, E., & Henie, M. (2016). Isolasi dan Uji Sensitivitas Merkuri pada Bakteri dari Limbah Penambangan Emas di Sekotong Barat Kabupaten Lombok Barat : Penelitian Pendahuluan Isolation and Mercury Sensitivity Test of Bacterias Isolated from Waste Disposal in Gold Mining Area in West S. *Proceeding Biology Education Conference*, *13*(1), 793–799.

[14] Hartline, R. (2023). *Microbiology Laboratory Manual*. Libretext. https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_Laboratory_Manual_(Hartline)/01%3A_Labs/1.18%3A_Catalase_Test

[15] Ginting, S. S. B., Suryanto, D., & Desrita, D. (2018). Isolasi Dan Karakterisasi Bakteri Potensial Probiotik Pada Saluran Pencernaan Ikan Bandeng (*Chanos chanos*). *Acta Aquatica: Aquatic Sciences Journal*.

[16] van Teeseling, M. C. F., de Pedro, M. A., & Cava, F. (2017). Determinants of bacterial morphology: From fundamentals to possibilities for antimicrobial targeting. *Frontiers in Microbiology*, *8*(JUL), 1–18. https://doi.org/10.3389/fmicb.2017.01264

[17] Vanawati, N., & Hirdibela, P. K. (2022). Uji Stabilitas Penyimpanan Ekstrak Tanaman Sari Ubi Jalar Ungu ( *Ipomoea batatas poiret* ) Sebagai Alternatif Pewarnaan Gram *Staphylococcus aureus*. *JAB-STABA*, *06*(02), 20–25.

[18] Lestari, N. W., & Budiharjo, A. (2016). Bakteri heterotrof aerobik asal saluran pencernaan ikan sidat (Anguilla bicolor bicolor) dan potensinya sebagai probiotik. *Bioteknologi*, *13*(1), 9–17.

[19] Irwansyah, I. (2018). Isolasi dan Identifikasi Bakteri Asam Laktat pada Saluran Pencernaan Ikan Bawal Bintang (*Trachinotus blochii*). *Intek Akuakultur*.

[20] André, A. C., Debande, L., & Marteyn, B. S. (2021). The Selective Advantage Of Facultative Anaerobes Relies On Their Unique Ability To Cope With Changing Oxygen Levels During Infection. In *Cellular Microbiology*.

[21] Mokoena, M. P. (2017). Lactic Acid Bacteria And Their Bacteriocins: Classification, Biosynthesis And Applications Against Uropathogens: A Mini-Review. In *Molecules* (Vol. 22, Issue 8). MDPI AG.

[22] Palma, V., Gutiérrez, M. S., Vargas, O., Parthasarathy, R., & Navarrete, P. (2022). Methods to Evaluate Bacterial Motility and Its Role in Bacterial–Host Interactions. *Microorganisms*, *10*(3), 1–14.

[23] Naveed, M., Nadeem, F., Mehmood, T., Bilal, M., Anwar, Z., & Amjad, F. (2021). Protease—A Versatile and Ecofriendly Biocatalyst with Multi-Industrial Applications: An Updated Review. *Catalysis Letters*, *151*(2), 307–323.

[24] Kurniasih, T., Lusiastuti, A. M., Azwar, Z. I., & Melati, I. (2014). Isolasi Dan Seleksi Bakteri Saluran Pencernaan Ikan Lele Sebagai Upaya Mendapatkan Kandidat Probiotik Untuk Efisiensi Pakan Ikan. *Jurnal Riset Akuakultur*, *9*(1), 99.

[25] Listiowati, E., Ekasanti, A., Nugrayani, D., Syakuri, H., Wisudyanti, D., Nurhafid, M., & Evander, Y. (2022). Studi Komunitas Bakteri Hidrolitik Saluran Pencernaan Ikan Nilem ( *Osteochilus vittatus* ) yang Dibudidayakan di Kabupaten Banyumas. *Jurnal Akuakultur Sungai Dan Danau*, *7*(2), 115–124.

[26] Solanki, P., Putatunda, C., Kumar, A., Bhatia, R., & Walia, A. (2021). Microbial Proteases: Ubiquitous Enzymes With Innumerable Uses. In *3 Biotech*.

[27] Diza, Y. H., Asben, A., & Anggraini, T. (2020). Isolasi, Identifikasi Dan Penyiapan Sediaan Kering Bakteri Asam Laktat Yang Berpotensi Sebagai Probiotik Dari Dadih Asal Sijunjung Sumatera Barat. *Jurnal Litbang Industri*. 10(2): 155-164.

[28] Gunkova, P. I., Buchilina, A. S., Maksimiuk, N. N., Bazarnova, Y. G., & Girel, K. S. (2021).

Carbohydrate Fermentation Test of Lactic Acid Starter Cultures. *IOP Conference Series: Earth and Environmental Science*.

[29] Ben Braïek, O., & Smaoui, S. (2019). Enterococci: Between Emerging Pathogens and Potential Probiotics. In *BioMed research international*.

[30] Alkalbani, N. S., Turner, M. S., & Ayyash, M. M. (2019). Isolation, Identification, And Potential Probiotic Characterization Of Isolated Lactic Acid Bacteria And In Vitro Investigation Of The Cytotoxicity, Antioxidant, And Antidiabetic Activities In Fermented Sausage. *Microbial Cell Factories*.

[31] Agustina, A., Saptiani, G., & Hardi, E. H. (2022). Isolation and identification of potential lactic acid bacteria as probiotics from the intestines of repang fish (*Puntioplites waandersi*). *AACL Bioflux*.

[32] Hossain, M. K., Iehata, S., Noordin, N. M., Kader, M. A., Emilia, S. N., Sung, Y. Y., & Abol-Munafi, A. B. (2022). Isolation And Identification Of Potential Host Probiotic Bacteria From Malaysian Mahseer, Tor Tambroides For Aquaculture Practices. *Biodiversitas*.