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*Full Length Article*

# Characteristics of Hydrolytic Exoenzyme Bacteria Isolated from the Intestines of Caught Jerbung Shrimp *Fenneropenaeus merguiensis*

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## Abstract

Jerbung shrimp (*Fenneropenaeus merguiensis* De Man 1888) is an important economic organism but little information is available about the composition of hydrolytic bacteria and lactic acid bacteria (LAB), especially on Jerbung shrimp. Bacteria that produce hydrolytic exoenzymes are very important to study due to their contribution to metabolism and industrial bioprocesses. This study aimed to analyze the proportion of hydrolytic bacteria and the presence of LAB in the digestive tract of Jerbung shrimp and to genetically characterize the enzyme-producing bacteria. The research was carried out by survey from two different waters, including the North Java Sea and the South Java Sea. Observation characteristics were carried out on hydrolytic bacteria for protein, starch, fat and cellulose. The proportions between activities provide an overview of how the tests differ from one another. Taxonomic classification based on 16S rRNA gene sequences in enzyme-producing isolates revealed that the fourteen isolates were grouped into four different bacterial classes. The order of Bacillales is the highly at 38.9%, followed by Lactobacillales, Gammaproteobacteria and Staphylococcales at 33.3, 17.0 and 11.1%, respectively. Measuring enzyme production using the agar diffusion technique shows that the presence of hydrolytic bacteria varies between enzyme types. There were the highest proportions of amylolytic bacteria at 75.3%, followed by proteolytic bacteria at 61.3, cellulolytic bacteria at 45.3 and lipolytic bacteria at 37.3%.

**Keywords:** Aquaculture; Bacterial communities; Jerbung shrimp intestines

## Introduction

Jerbung shrimp have been recorded in several marine waters in Indonesia as a local name of *Fenneropenaeus merguiensis* (De Man 1888), including the south and north coasts of Java, the Strait of Malacca, South Kalimantan, Bintuni Bay and the Arafura Sea (Soegianto and Hamami 2007; Silaen and Mulya 2018; Suryanti *et al.* 2018). The use of Jerbung shrimp is limited to consumption either domestically or for export purposes. Currently, studies regarding the characteristics of Jerbung shrimp are still limited, especially regarding the characteristics of microbes in the digestive system of Jerbung shrimp caught in marine waters. As the largest ecosystem, marine waters are one of the richest reserves of bacteria, most of which remain unexplored and unexploited. This very large population of bacteria is considered a potential source of new enzymes and the many bioactive compounds they contain (Paul *et al.* 2021).

The gastrointestinal (GI) tract hosts complex bacterial communities that significantly influence shrimp physiology through metabolic interactions (Thursby and Juge 2017). Producing a variety of extracellular hydrolytic enzymes, the intestinal flora carries out a variety of different catabolic and biotransformation reactions. Interactions in the intestine

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occur with complex mechanisms because various factors influence both internally and externally (Chelakkot *et al.* 2018). One of the internal factors that influence activity in the intestine is digestive enzymes. Then the external factor that influences intestinal activity is the type of feed (Wu *et al.* 2012; Nurhafid *et al.* 2021). Jerbung shrimp are shrimp that have eating habits from the omnivore group, tending to be carnivorous (Kusna *et al.* 2019). Several studies have found that Jerbung shrimp eat unidentified animal fragments and litter in their intestines. However, the eating habits of Jerbung shrimp are omnivores. Habits and type of feed can influence enzyme activity in the shrimp intestine (Meager *et al.* 2005; Vance and Rothlisberg 2020). Additionally, it was explained that bacteria in shrimp digestive tracts are an interesting characteristic to study.

Bacteria are the most common microorganisms that found in the intestines, including shrimp intestines (Hou *et al.* 2018). Most of the bacteria in the intestine are bacteria that come from outside or are not native bacteria that live in the intestine. Bacteria in the intestine will continuously change depending on conditions in the intestine (Hou *et al.* 2020). The type of feed consumed is a factor that greatly influences the digestive bacterial community (Nurhafid *et al.* 2021). Several studies have shown that the type of feed will influence the structure and community of bacteria. Several bacteria with certain abilities have been widely studied in the intestines of aquatic biota including shrimp (Gao *et al.* 2019; Fitriadi *et al.* 2023a, b). For example, bacteria can degrade organic feed materials such as protein and amylum so that the feed absorption process can be carried out efficiently (Fitriadi *et al.* 2023a, b; Ayal *et al.* 2024). In addition, aquatic organisms have different numbers and proportions of bacteria that degrade organic matter in digestion (Sinatryani *et al.* 2014). Since the digestive tract is an open system that continuously interacts with the surrounding environment, diverse bacterial populations in marine environments, taking advantage of their uninterrupted access to the digestive tract make up the bulk of the GI microbiota (Mondal *et al.* 2010). Therefore, studying shrimp GI bacteria is essential for the discovery of beneficial strains as promising sources of new biocatalysts.

This study evaluated the hydrolytic capacity of GI bacteria isolated from the digestive tract of Jerbung shrimp as potential tools for aquaculture and biotechnology. The isolated strains were examined for the production of four different hydrolytic enzymes such as protease, lipase, cellulase, amylase, and the enzyme-producing strains were characterized based on the 16S rRNA gene sequence.

## Materials and Methods

### Research sample

Samples were taken from the entire intestine of the Jerbung shrimp from the foregut, midgut, and the hindgut. Jerbung shrimp as a sample are shrimp caught from different water locations, namely the north coast waters, in this case, Pemalang, and the south coast waters, in this case, Cilacap. Jerbung shrimp samples were grouped into three categories with different average lengths and weights, namely large (22.2 ± 1.6 cm) (92.95 ± 10 g) medium (16.62±1.1 cm) (39.92±11 g) and small (12.12±0.2 cm) (18.60±1.6 g). A total of four samples were taken from each size category group for direct analysis at the sampling location.

### Inoculation and isolation of intestines bacteria

Three representative shrimp from each group were suspended in 10 mL of sterile seawater for bacterial inoculation. Then, the sample suspension was subjected to serial dilution up to 4 times. 100 $\mu$L of each dilution was taken and spread on the surface of the MRSA (De Man Rogosa and Sharpe Agar) growth medium which had been added with 2% $CaCO_3$ (Calcium Carbonate). Inoculum samples were incubated at 30ºC for 48 h. Random samples were taken of 25 isolates to be purified and selected for their ability to produce lactic acid which was indicated by the presence of a clear zone on the MRSA medium which was added with 2% $CaCO_3$.

### Bacterial hydrolytic

The isolated isolate from each sample was tested on specific media to detect bacterial enzyme activity. Several enzymes tested in this research (Bairagi *et al.* 2002; Fitriadi *et al.* 2023a, b) are protease, amylase, lipase and cellulase. Several detection media for each enzyme activity can be done by a). Protease enzyme detection was conducted by adding 2% skim milk to the growth medium. b). Detection of the amylase enzyme was conducted by adding 1% tapioca flour to the growth medium and dropping Lugols iodine at the end of the observation. c). Lipase enzyme detection was conducted by adding 1% Tween 80 and 0.05% phenol red to the growth medium. d). Cellulase enzyme detection was conducted by adding 1% CMC to the growth medium and dropping congored with concentration 0.1 g/100 mL aquades at the end of the observation. Purified inoculated isolates were tested for enzymatic hydrolysis activity by culturing on different enzyme media. Next, it was incubated for 48 h at 30ºC. Then the enzyme activity was observed by measuring the clear zone formed (Fitriadi *et al.* 2023a, b).

### Proportion of hydrolytic bacteria

Bacterial isolates tested for each type of enzyme are grouped according to their proportion. Bacterial proportions were conducted to see differences in types of bacterial enzyme activity. The proportion of hydrolytic bacteria was calculated using the following formula:

$$\text{Proportion of hydrolytic bacteria (\%)} = \frac{\text{Number of hydrolytic bacteria}}{\text{Number of bacteria isolated}} \times 100$$

**Molecular identification**

The molecular identification of bacteria is based on their ability to hydrolyze organic compounds with clear zones. In the initial stage of identification, selected isolates were cultured in liquid media, namely Tryptone soy broth, and incubated for 48 h at 30ºC. Next, the bacteria were extracted using a SkyFire Biotech automatic nucleic acid extractor machine using the Skyfire Magnetic Beads method kit with a modified procedure. The cultured bacteria were centrifuged and washed using sterile free water three times. Then the lysis stage was carried out using a lysis reagent from Transgene Biotech by heating at 70ºC for 30 min. The extracted DNA was then amplified using conventional PCR. The PCR kit used in amplification was HS RedMix 2x from Bioline with universal primers 27F (5'- AGA GTT TGA TCC TGG CTC AG -3') and 1492R (5'- GGT TAC CTT GTT ACG ACT T -3') using the procedure according to the kit requirements (https://www.bioline.com/mwdownloads/download/link/id/2687/mytaq_hs_red_mix_product_manual.pdf) with an annealing temperature of 55ºC. The PCR product was visualized using agarose gel electrophoresis with a product yield of 1500 bp (Marchesi *et al.* 1998). Then the Sanger method is used to read samples that have been amplified successfully. Sequences were analyzed using the NCBI online program (https://www.ncbi.nlm.nih.gov/) to be blasted to determine the identity of the sample.

**Data analysis**

Data from the research were analyzed descriptively to describe the characteristics of hydrolytic bacteria in the Jerbung shrimp intestines. The hydrolytic activity of bacteria is calculated as a percentage of all isolated isolates. Several isolates were identified to determine the identity of the isolate and to compare the characters with several references. In conclusion, the presence of hydrolytic bacteria in shrimp intestines indicates that the shrimp is ready for cultivation.

## Results

Shrimp samples were taken from two different locations, namely the North Java Sea and the South Java Sea (Fig. 1). The shrimp samples obtained were then grouped based on size, such as large, medium, and small. The shrimp samples were taken from each size group and crushed to form one size group by combining the samples in a container. The shrimp size groups used as samples are presented in Table 1.

Observing the morphological characteristics of colonial growth on MRSA was used to determine the level of intestinal bacterial infection. Additionally, the process of taking bacterial isolates was random, with 25 isolates per size group. Random isolates were taken to test bacterial enzyme activity on several different media to detect the ability of bacteria to produce enzymes. The types of enzymes detected in intestinal bacteria include protease, amylase, lipase, and cellulase and the proportion of each enzyme produced is calculated. The proportion of Jerbung shrimp intestines bacteria is shown in Table 2.

Amylolytic bacteria show the highest proportion of hydrolytic bacteria, followed by proteolytic, cellulolytic, and lipolytic bacteria. This is related to the habitat and eating habits of shrimp. The sample shrimp in the study were caught shrimp so that in an environment free of organic materials, starch, and protein were higher than lipase and cellulase, thus affecting the presence of bacteria. The proportion of LAB that produces enzymes was examined specifically by observing LAB isolates. Based on the results of observations, shows that LAB isolates are generally fewer than non-LAB bacteria. The results of the study showed that the proportion of LAB isolates obtained had an average value of 8%. The highest proportion of LAB isolates was found in medium size Jerbung shrimp (16%), followed by large size (8.4%) and small size (4.0%). Data can be seen in Table 3.

Several samples of the intestine's bacteria of Jerbung shrimp were successfully identified using the 16S rRNA gene. The identification results of some of the isolates showed that the identity of the bacteria obtained came from the Bacillales, Lactobacillales, Staphylococccacles and Gammaproteobacteria order with an identified proportion of 38.9, 33.3, 37.0 and 11.1% (Fig. 2). Based on identification results, the intestines bacteria of the Jerbung shrimp are dominated by *Bacillus* spp. which is non-LAB. However, in this study, bacteria from the LAB group were also found. The results of the identification of hydrolytic bacteria isolated from the intestines of Jerbung shrimp can be seen in Table 4 and proportion of identified bacteria in Fig. 2.

Based on potential bacteria, analysis shows that most of the intestinal bacteria of Jerbung shrimp have the ability to hydrolyze several organic compounds at once. It has been shown that *B. cereus* strain GS14.2 can hydrolyze proteins, starches, fats, and cellulases, in addition to carbohydrates. Not only that, but this strain also shows the ability to inhibit pathogens, namely *V. alginolyticus* and *V. parahaemolyticus*. Furthermore, most of the bacterial isolates from the LAB group exhibited high enzyme activities and capabilities. However, the LAB isolates found in this study can inhibit the growth of several types of *Vibrio*. Some of the strains in Table 5 represent only a small portion of the total population. This data indicates that most bacteria have the ability to support the digestion of the Jerbung shrimp.

## Discussion

Shrimp is an export commodity with promising economic value. It is a priority commodity to meet international market needs (Amin *et al.* 2022; Abuzar *et al.* 2023). As an archipelagic country, Indonesia has a good opportunity to

**Table 1:** Location and sample size group

| Sample Location | Size | | | | | |
|---|---|---|---|---|---|---|
| | Large | | Medium | | Small | |
| | Weight (g) | Length (cm) | Weight (g) | Length (cm) | Weight (g) | Length (cm) |
| North sea of Java | 92.95±10 | 22.00±1.6 | 39.92±11 | 16.62±1.1 | 18.60±1.6 | 12.12±0.2 |
| South sea of Java | 81.60±12 | 22.30±0.9 | 33.40±1.4 | 17.50±0.3 | 18.97±1.6 | 14.62±0.5 |

**Table 2:** Proportion of hydrolytic bacteria in the intestine of Jerbung shrimp

| Location | Size | Isolates of Total Bacteria | Enzymatic of total bacteria (%) | | | |
|---|---|---|---|---|---|---|
| | | | Proteolytic | Amylolytic | Lypolytic | Cellulolytic |
| North sea of Java | Large | 25 | 48 | 80 | 16 | 12 |
| | Medium | 25 | 48 | 92 | 68 | 60 |
| | Small | 25 | 80 | 96 | 32 | 40 |
| South sea of Java | Large | 25 | 52 | 88 | 36 | 56 |
| | Medium | 25 | 84 | 76 | 24 | 40 |
| | Small | 25 | 56 | 20 | 48 | 64 |
| Average | | | 61.3 | 75.3 | 37.3 | 45.3 |

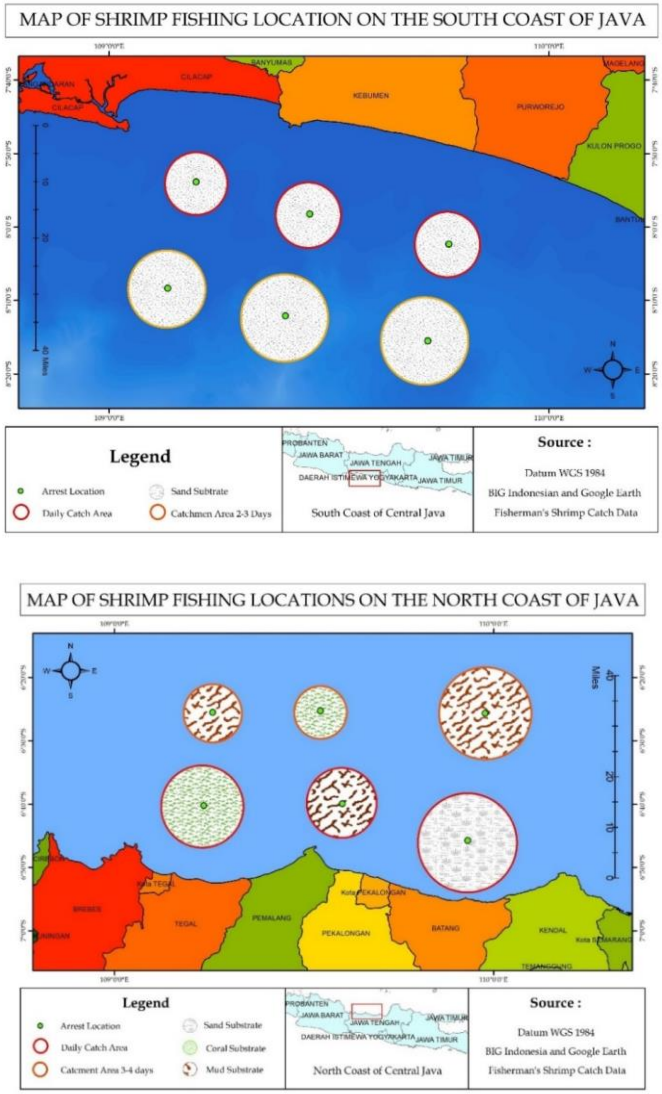


**Fig. 1:** Jerbung shrimp fishing location. (**A**) Fishing locations in Pemalang waters which have sandy, muddy or slightly rocky characteristics; (**B**) Fishing locations in Cilacap waters which have sandy water characteristics

**Table 3:** Proportion of LAB

| Location | Size | Isolates of Total Bacteria | Proportion of LAB (%) |
|---|---|---|---|
| North sea of Java | Large | 25 | 8 |
| | Medium | 25 | 16 |
| | Small | 25 | 0 |
| South sea of Java | Large | 25 | 4 |
| | Medium | 25 | 16 |
| | Small | 25 | 4 |
| Average | | | 8 |

**Table 4:** Identification of hydrolytic bacteria in the intestines of Jerbung shrimp

| Isolate code | Idebtified organism | Identity (%) | Type of Bacteria | Order | Acc. Number | Location |
|---|---|---|---|---|---|---|
| GL22 | *Bacillus anthracis* | 99 | Non-LAB | Bacillales | KY649419.1 | North sea of Java |
| RL20 | *Bacillus subtilis* | 100 | Non-LAB | Bacillales | ON892497.1 | South sea of Java |
| KL13 | *Staphylococcus hominis* | 95 | Non-LAB | Staphylococcales | MK910112.1 | North sea of Java |
| UM16 | *Bacillus cereus* | 99 | Non-LAB | Bacillales | EF633184.1 | North sea of Java |
| RM13 | Enterococcus hirae | 100 | LAB | Lactobacillales | MK202998.1 | South sea of Java |
| GS14.1 | *Bacillus pseudomycoides* | 95 | Non-LAB | Bacillales | MK574858.1 | North sea of Java |
| RS22 | *Staphylococcus pasteuri* | 100 | Non-LAB | Staphylococcales | MT539733.1 | South sea of Java |
| GS14.2 | *Bacillus cereus* | 97 | Non-LAB | Bacillales | KU898278.1 | North sea of Java |
| GM23 | Enterococcus hirae | 96 | LAB | Lactobacillales | KX752873.1 | North sea of Java |
| GS14.3 | *Bacillus pseudomycoides* | 98 | Non-LAB | Bacillales | MK574858.1 | North sea of Java |
| RS19.1 | *Bacillus* sp. | 99 | Non-LAB | Bacillales | MG470747.1 | South sea of Java |
| UM.12 | *Psychrobacter celer* | 99 | Non-LAB | Gammaproteobacteria | MK512026.1 | North sea of Java |
| UM 22 | *Psychrobacter celer* | 99 | Non-LAB | Gammaproteobacteria | MK205165.1 | North sea of Java |
| GM 19 | *Psychrobacter celer* | 99 | Non-LAB | Gammaproteobacteria | MK995612.1 | North sea of Java |
| J04 | *Weissella confusa* | 99 | LAB | Lactobacillales | MT473367.1 | South sea of Java |
| J00 | *Weissella confusa* | 100 | LAB | Lactobacillales | MT613537.1 | South sea of Java |
| J07 | *Weissella confusa* | 97 | LAB | Lactobacillales | LC274616.1 | South sea of Java |
| J71 | *Enterococcus hirae* | 100 | LAB | Lactobacillales | MW674392.1 | South sea of Java |

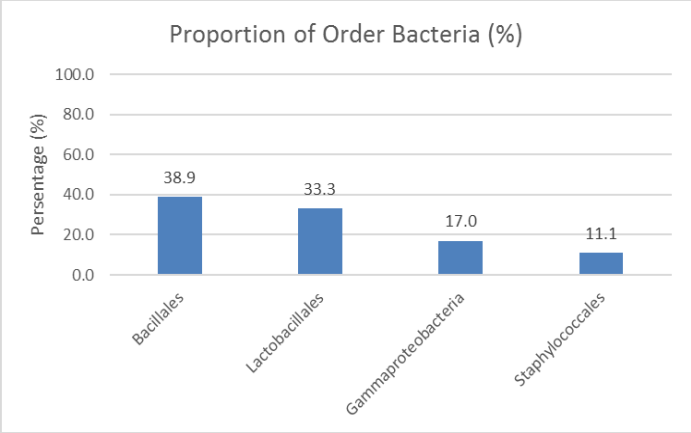


**Fig. 2:** Proportion of Identified Bacteria

develop the fisheries sector. Jerbung shrimp are known to have widespread in almost all the archipelagos in Indonesia (Wiradana *et al.* 2020). Even though the distribution is wide, to support the need for shrimp exports we cannot always rely on catches, hence it needs to be developed through cultivation.

Based on the studies that have been carried out, Jerbung shrimp should be able to be cultivated by society because shrimp can be seeded on a lab scale so that it can meet the needs of larvae (Suning *et al.* 2022).Based on the results of the study, it shows that the intestinal bacteria of Jerbung shrimp have varying characteristics of the presence of hydrolysis bacteria. It is very common that bacteria in the intestine have varying proportions and dynamic existence (Li *et al.* 2014). The most dominating factor in this case is the amount of organic material (Liu *et al.* 2016). The presence and type of organic material can influence the number and type of bacteria (Wu *et al.* 2012). This is the basis for estimating the findings in this research. Bacteria that have the activity of hydrolyzing starch in research show the highest percentage, followed by protein, fiber and fat (Artha *et al.* 2019). The high presence of amylolytic bacteria

**Table 5:** Potential of hydrolytic bacteria in the intestines of Jerbung shrimp

| Isolate code | Strain | Hydrolytic activity | | | | Anti-vibrio | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pro | Amy | Lyp | Cell | VA | VH | VN | VP |
| GL22 | *Bacillus anthracis* | + | ++ | - | - | - | - | - | - |
| RL20 | *Bacillus subtilis* | - | ++ | + | + | + | - | + | - |
| KL13 | *Staphylococcus hominis* | - | - | ++ | - | - | - | - | - |
| UM16 | *Bacillus cereus* | ++ | ++ | ++ | - | - | - | - | - |
| RM13 | *Enterococcus Hirae* | ++ | ++ | ++ | ++ | + | ++ | - | + |
| GS14.1 | *Bacillus pseudomycoides* | + | + | +++ | + | - | - | - | + |
| RS22 | *Staphylococcus pasteuri* | ++ | + | - | + | - | - | - | - |
| GS14.2 | *Bacillus cereus* | + | ++ | +++ | + | - | + | + | + |
| GM23 | *Enterococcus Hirae* | +++ | - | - | +++ | ++ | + | - | + |
| GS14.3 | *Bacillus pseudomycoides* | ++ | +++ | ++ | + | - | - | - | + |
| RS19.1 | *Bacillus* sp. | + | - | - | - | + | - | - | + |
| UM.12 | *Psychrobacter celer* | ++ | +++ | + | - | + | - | - | - |
| UM 22 | *Psychrobacter celer* | ++ | ++ | - | - | - | - | - | - |
| GM 19 | *Psychrobacter celer* | - | - | + | - | - | + | - | - |
| J04 | *Weissella confusa* | - | +++ | +++ | +++ | ++ | ++ | - | + |
| J00 | *Weissella confusa* | - | - | +++ | +++ | - | - | - | + |
| J07 | *Weissella confusa* | - | +++ | +++ | ++ | ++ | - | - | + |
| J71 | *Enterococcus hirae* | ++ | ++ | ++ | +++ | ++ | + | - | + |

Description: Pro = proteolytic, amy = amylolytic lyp = lypolytic, cell = cellulolytic, VA = *V. alginolyticus*, VH = *V. harveyi*, VN = *V. navarensis*, VP = *V. parahaemolyticus*, Weak (+); Moderate (++); Strong (+++); No activity (-)

is thought to be because the Jerbung shrimp used for research were caught shrimp so the feed consumed was varied with low protein. Previous studies showed that the results of intestines dissection to observe the type of feed consumed by bluefin shrimp were pieces that were not identified (Vance and Rothlisberg 2020). Then, if it is related to the habits of Jerbung shrimp which are included in the omnivore category, the variety of feed consumed comes from carbohydrate sources (Liu *et al.* 2016). This suspicion can be related to the habitat of the Jerbung shrimp, in certain phases the Jerbung shrimp inhabit mangroves so they consume more feed with a high carbohydrate content (Meager *et al.* 2005; Vance and Rothlisberg 2020). It will be different if the shrimp are carnivorous and the intestines are dominated by bacteria that are proteolytic (Bairagi *et al.* 2002; Nurhafid *et al.* 2021).

The proportion of LAB in this study was found to be 8% of the total bacteria obtained. The proportion value of LAB in the intestines of Jerbung shrimp does not appear to dominate the shrimp intestines. This is because LAB is a bacteria that is not native to the intestines of shrimp or aquatic animals (Ringø *et al.* 2020). LABs are bacteria from terrestrial animals that enter the intestines of aquatic animals on a temporary basis so it is very unlikely that the presence of LAB dominates the shrimp intestines (Verschuere *et al.* 2000; Di Gioia and Biavati 2018). This research study found that the proportion of LAB was relatively low from the total bacterial population identified. But the role of LAB in the digestive tract is very important; this has been explained by several previous studies. For example, research (El-Naby *et al.* 2020; Arif *et al.* 2020) explains that Gram-positive lactic acid-producing bacteria influence most of the normal intestinal microbiota in humans and animals. Typically, LAB are rod-shaped bacteria and do not form spores (El-Shall *et al.* 2020). The nutritional requirements are complex, and are fermentative, anaerobic or aerotolerant and acidophilic or aciduric (Alagawany *et al.* 2018; El-Aziz *et al.* 2020). In addition, LAB are found in various habitats, especially carbohydrate-rich substrates such as animal and human mucous membranes, plants or other materials derived from plants and waste (Vrese and Schrezenmeir 2008; Naiel *et al.* 2020). Shrimp productivity is closely linked to health, and the intestinal microbiome is increasingly recognized as an important driver of aquaculture success. The microbes that inhabit the intestines, commonly referred to as intestinal microbiota or intestinal microbiome, interact with their host and contribute to a number of key host processes, including digestion and immunity.

Based on the results of this study, it shows that the bacterial isolates obtained mostly came from the *Bacillus* spp. group. Most studies on intestines bacteria in shrimp and other aquatic animals show that the *Bacillus* group is a common bacteria found in all environments (Kour *et al.* 2019; Soltani *et al.* 2019; Ginting *et al.* 2021). This group of bacteria is interesting because of their abilities and roles, which are mostly beneficial to the host and the environment (Thurlow *et al.* 2019; James *et al.* 2021). Furthermore, another isolate was identified from the LAB group which is not native to shrimp intestines bacteria because this group of bacteria is a group of bacteria that originates from the digestion of terrestrial animals. However, most of this group also has good abilities in helping the digestion of aquatic animals (Mohamad *et al.* 2020). Based on the discovery of isolates identified on hydrolytic enzymes, it can be concluded that the bacteria isolated in this study are bacteria that have an important role in the intestines of the Jerbung shrimp. Moreover, if it is related to the intestinal condition of the caught Jerbung shrimp, it can indicate that Jerbung shrimp can be domesticated. The presence of these

hydrolytic bacteria can help the intestines effectively break down feed compounds provided in future cultivation. Numerous studies have shown most aquaculture probiotics come from *Bacillus* and LAB bacteria, which are sources of probiotics (Olmos 2014; Wanna *et al.* 2021).

## Conclusion

Hydrolytic bacteria showed varying proportions between enzyme types. Most of the bacterial groups from the identification results came from the Bacillales order and the proportion of LAB obtained from the shrimp digestive tract was around 8%. According to this study, the digestive tract of the Jerbung shrimp contains a diverse bacterial population that produces extracellular hydrolytic enzymes such as protease, amylase, lipase, and cellulase. The enzyme production capacity of the isolates suggests that the intestinal bacterial flora serves as an important source of digestive enzymes, metabolism and host nutrition. Isolates that showed high activity in this study require further investigation to understand their actual capacity to produce hydrolytic enzymes that could lead to aquaculture applications.

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## Conflicts of Interest

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

## Data Availability

Not applicable.

## Ethics Approval

Not applicable.

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