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Activity of Cellulolytic Bacteria Isolated from the Digestive Tract of Jerbung Shrimp (*Fenneropenaeus marguensis*)

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Activity index, Cellulolytic bacteria, Digestive tract, Jerbung shrimp

Abstract

Bacteria found in the shrimps' digestive tract mostly play a major role in nutrient absorption. One of the nutritional contents that is quite difficult for shrimp to absorb is fiber. Bacteria that can degrade fiber content are known as cellulolytic bacteria. This study aimed to determine the activity index of cellulolytic bacteria found in the shrimp's digestive tract. The research method includes sampling shrimp with three different categories based on average length and weight, divided into three categories such as large (22.2 g ± 87.3 cm), medium (17.1 g ± 36.7 cm), and small (13.4 g ± 18.8 cm) taken from the north coast waters of Pemalang and the south coast waters of Cilacap. Furthermore, bacterial isolation, morphological observation, calculation of bacterial abundance, and testing of cellulolytic activity were carried out. The results showed that 150 bacteria were successfully isolated from both locations at different sizes, and a total of 68 bacterial isolates had cellulolytic activity. There were 22 potential isolates from each coastal water that had a great cellulolytic activity index, ranging from 1.4 to 7.0. Moreover, the most potential activities were performed by 8 bacterial isolates encoded with GM.1, GM.2, GM.3, GM.4, GM.5, GM.6, GM.7, and GM.8 with a range of cellulolytic index obtained ranging from 3.0-7.0. The high activity index of the cellulolytic bacteria obtained is a fairly promising potential as a probiotic candidate that plays a role in degrading fiber in food found in the digestive tract of shrimp so that it can accelerate its growth.

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INTRODUCTION

Jerbung shrimp is a species caught in Indonesian sea waters that has high economic value. This shrimp is quite sought after by the public because it has a delicious taste, large size, and high export value (Zacharia and Kakati, 2004). In Indonesia, jerbung is found in several sea waters, including Java, Sumatra, Kalimantan, and Maluku (Suryanti *et al*., 2018). Jerbung shrimp in each region of Indonesia has a different local name, such as kelong shrimp, pate shrimp, popet shrimp, sharp shrimp, and white shrimp (Mulya and Yunasfi, 2018). The advantages of jerbung shrimp in sea waters are that they have fast growth and are resistant to disease compared to vannamei shrimp (*Litopenaeus vannamei*) (Widiani *et al*., 2021; Wiradana *et al*., 2020). The rapid growth and resistance to disease are largely assisted by the microflora found in the digestive tract (Widanarni *et al*., 2012).

The microflora found in the digestive tract are mostly bacteria originating from the environment and not native bacteria that live in the intestines (Senghor *et al*., 2018). Intestinal microflora plays an important role in stimulating metabolic function, improving digestion (Holt *et al*., 2021), increasing immune responses, and protecting against exogenous bacteria and diseases (Imaizumi *et al*., 2021; Ringø *et al*., 2022). Changes in the microflora found in the intestines can also affect the diet of aquatic animals. Changes in the diet of aquatic animals are influenced by the activity of extracellular enzymes produced by bacteria found in the digestive tract (Augustine and Joseph, 2018). The ability of intestinal microflora to produce extracellular enzymes for substrate degradation is important to study to understand the nutritional needs and physiology of the host organism (Tanu *et al*., 2012).

Extracellular enzymes are secondary metabolites produced by bacteria. One of the extracellular enzymes produced by bacteria is the cellulase enzyme. The cellulase enzyme plays a role in hydrolyzing the β-1,4 glycoside bonds in cellulose to produce glucose (Lokapirnasari *et al*., 2015). Cellulase enzymes are generally widely used in the food technology, textile, animal feed, paper, agriculture, and research development industries (Irvan *et al*., 2019). In fish farming, the cellulase enzyme plays an important role in reducing the fiber contained in the feed to increase the digestibility of shrimp (Sari *et al*., 2017). In addition, the degradation of cellulose in fish feed raw materials can increase fish growth (Zhou *et al*., 2013). Some genera that produce cellulase enzymes include *Clostridium, Mycobacterium, Aeromonas, Acinetobacter, Bacillus, Buttiauxellia, Enterococcus, Enterobacter, Lactococcus, Microbacterium, Ralstonia, Serratia, Shewanella,* and *Sphingomonas* (El-Sheikh, 2003).

The ability of cellulolytic bacteria to degrade cellulase can be seen from the activity index produced. The higher activity index of the cellulolytic enzyme produced indicates the potential of cellulolytic bacteria to degrade cellulose (Demissie *et al*., 2024). Research on the activity index has been widely conducted from shrimp intestines, sediments, and pond water. For example, in the study of Ren *et al*. (2020) obtained 58 strains of cellulolytic marine bacteria from the intestines of sea shrimp with an activity index range of 1.1 - 4.0. Furthermore, Dewiyanti *et al*. (2022a) obtained 39 isolates of cellulase bacteria from the mangrove environment with a cellulolytic index ranging from 0.31 to 4.82. The cellulolytic activity index produced by bacteria correlates with the availability of cellulase in shrimp food or the shrimp's environment. This is the basis for this research, where a study of the cellulolytic bacterial activity index in shrimp needs to be conducted to determine how much cellulolytic bacterial activity is found in the digestive tract of shrimp isolated from different locations on the north and south coasts of Java Island.

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METHODOLOGY
Ethical Approval

All samples were gathered with owner consent and used according to the ethical guidelines provided by the Health Research Ethics Committee, UNSOED Faculty of Health Sciences with an approved ID of No: 1613/EC/KEPK/X/2024.

Place and Time

The research was conducted from January to February 2024. A sampling of jerbung shrimp (*Fenneropenaeus merguiensis*) was collected from the waters of the northern coast of Pemalang and the waters of the southern coast of Cilacap. Isolation of bacteria to testing of cellulase enzyme activity was carried out at the Research Laboratory of the Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University.

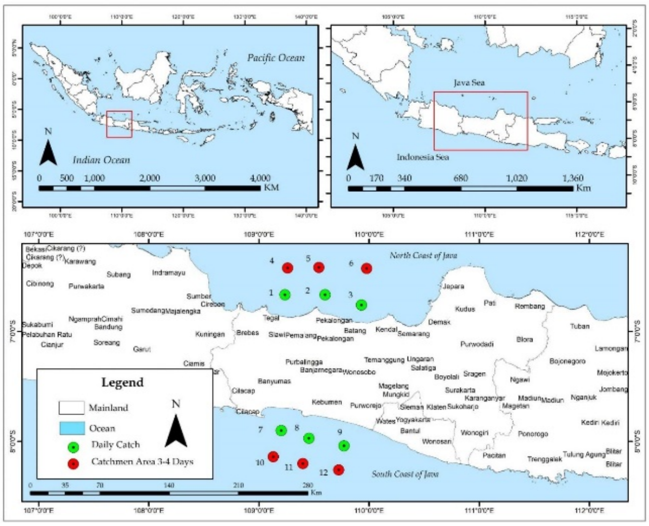

Figure 1. Research Location.

Research Materials

The tools used in this study include Petri dishes, a measuring cup 5 ml, test tube, object glass, tube rack, Erlenmeyer 250 mL, pipette pump, measuring pipette, aluminum foil, surgical tools, plastic wrapping, ose needle, vortex, bunsen, lighter, analytical balance, micropipette 100-1000 μL, microtip 1250 μL, microtube 2 mL, box tip, autoclave, push term, stationery, tissue, cool box, Vertical laminar airflow (LAF), hot plate magnetic stirrer, colony counter, and incubator. The materials used in this study include tryptic soy agar media (Merck), carboxymethyl cellulose, alcohol 70%, alcohol 96%, physiological solution (NaCl 0.9%), distilled water solution, and spiritus.

Research Design

The samples used in this study were jerbung shrimp. A total of 24 jerbung shrimp used in this study were captured from different water locations, namely north coast waters, in this case, Pemalang, and south coast waters, represented by Cilacap (Figure 1). Samples were grouped into three categories with different average lengths and weights: large (22.2 g ± 87.3 cm), medium (17.1 g ± 36.7 cm), and small (13.4 g ± 18.8 cm). Samples of 4 shrimp in each category collected from both water sites were stored in a cool box for further processing in the laboratory.

Work Procedure
Bacteria Isolation

Shrimp samples 4 from different size categories were dried using tissue and weighed. Furthermore, the shrimp were sprayed with 96% alcohol to kill

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microorganisms attached to the shrimp carapace. The shrimp digestive tract was aseptically dissected and then placed in a mortar for homogenization. The smooth shrimp digestive tract was then isolated. The bacterial isolation process began by performing serial dilutions on the shrimp digestive tract samples. Sample dilution was carried out by weighing 0.1 g of shrimp digestive tract samples suspended in 0.5 mL of physiological solution and then homogenized with a vortex. The dilution process was carried out using three test tubes containing 4.5 mL of sterile physiological (dilution 10^{-1}-10^{-3}). A sample of 0.5 mL was taken and homogenized with 4.5 mL of physiological solution in the first tube (dilution 10^{-1}). A total of 0.5 mL of sample suspension was taken from the first tube and homogenized in the second tube (10^{-2} dilution), and this procedure was carried out until the third tube (10^{-3} dilution). The results of the 10^{-1} to 10^{-3} dilutions were taken as much as 0.5 mL each and cultured using the Spread plate method on Tryptic Soy Agar (Millipore) media. Incubation was carried out for 18-24 hours at a temperature of 28ºC (Nurhafid *et al*., 2021).

Total Bacterial Abundance

The bacterial colonies that grew on the surface of TSA agar from dilution 10^{-1} to dilution 10^{-3} were counted in their entirety, then the abundance of bacteria in the digestive tract of jerbung shrimp was calculated using the total plate count (TPC) calculation method with a colony number of 30-300 (Riady *et al*., 2024). The calculation of bacterial abundance can be calculated using the Madigan and Martinko (2006) formula, which is modified as follows:

$$\text{Number of bacteria } \frac{\text{CFU}}{\text{g}} = \text{Number of colonies x } \frac{1}{\text{Dilution}} \text{ x } \frac{1}{\text{Culture volume}} \text{ x } \frac{1}{\text{Sampel weight}}$$

Observation of Bacterial Morphology

Bacteria growing on the surface of TSA media were observed for their macroscopic morphological characteristics consisting of color, shape, elevation, edge, size, and texture (Hikmawati *et al*., 2019). Approximately 25 isolates were obtained from each dilution of the sample by growing separate colonies. Bacterial colonies were taken based on visible differences in morphology. After selecting the bacteria, TSA media were used to stock them using the streak plate technique.

Cellulolytic Activity Testing

Cellulolytic bacteria were tested on TSA media supplemented with 1% carboxymethyl cellulose (CMC) to produce cellulase enzymes (Artha *et al*., 2019). The test was carried out using the dotting method where the bacterial colony in the stock was taken as much as one ose then scratched on the CMC media and incubated for 48 hours at a temperature of 28°C (Kurniawan *et al*., 2019). Bacterial isolates that have cellulolytic activity are characterized by the formation of a clear zone around the colony. Visualization of the clear zone that is formed can be seen after being dripped with Congo red solution (Gupta *et al*., 2012). The results of the clear zone formed are measured by the diameter of the bacterial colony and the diameter of the clear zone to determine the index of cellulolytic activity formed (Dewiyanti *et al*., 2022b). The results of these measurements are included in the cellulolytic activity calculation formula as quantitative data. The formula for calculating the cellulolytic activity index refers to the formula used by Bradner *et al*. (1999) as follows:

$$\text{Cellulolytic Activity} = \frac{\text{Total diameter of clear zone} - \text{diameter of bacterial colony}}{\text{diameter of bacterial colony}}$$

The proportion of cellulolytic bacteria was calculated using the Sinatryani, (2014) formula:

$$\text{Proportion of cellulolytic bacteria\%} = \frac{\text{the number of cellulolytic bacterial colonies obtained}}{\text{The total number of colonies observed}} x100$$

Data Analysis

The data obtained regarding bacterial abundance, colony morphology, cellulolytic activity index, and proportion of cellulolytic bacteria were then analyzed statistics and presented in the form of figures, tables, and

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graphs. Therefore, the data were analyzed descriptively and compared with the literature.

RESULTS AND DISCUSSIONS

The Number of Bacterial Abundance

The abundance of bacteria in the digestive tract of jerbung shrimp showed quite varied values for each shrimp size. In the coastal waters of Pemalang, the highest bacterial abundance was found in small shrimp, which was 1.5 x 10^7 CFU/g, followed by medium size (1.4 x 10^7 CFU/g) and large size (1.0 x 10^6 CFU/g). Meanwhile, in the waters of Cilacap, the highest abundance of shrimp was found in medium size shrimp, which was 2.1 x 10^6 CFU/g, followed by large size (2.2 x 10^5 CFU/g) and small size (9.3 x 10^5 CFU/g).

There was a difference in the abundance of bacteria in the digestive tract of shrimp isolated from the coastal waters of Pemalang and Cilacap, where the abundance value of bacteria in the digestive tract of shrimp isolated from the coastal waters of Pemalang was higher than the abundance of bacteria in the coastal waters of Cilacap. The abundance of bacteria in the digestive tract of jerbung shrimp can be seen in Table 1.

Table 1. Abundance of digestive tract bacteria of jerbung shrimp isolated from the coastal waters of Pemalang and Cilacap.

Shrimp Size	The number of bacterial abundance (CFU/g)	
	Pemalang	Cilacap
Small	1.5 x 10^7	2.2 x 10^5
Medium	1.4 x 10^7	2.1 x 10^6
Large	1.0 x 10^6	9.3 x 10^5
Average	1.0 x 10^7	1,1 x 10^6

Bacterial Morphology

The morphology of bacterial colonies obtained in this study tended to vary greatly. There were 150 bacterial isolates obtained, which were classified into 106 types. Differences in bacterial morphological characteristics were taken according to Bergey's Manual of Determinative Bacteriology Book (Holt *et al.*, 1994), which includes shape, elevation, edge, color, size, and texture. In this study, the colony shapes obtained were circular, irregular, phyllomantous, rhizoid, and spindle. Elevation consisted of convex, crateriform, flat, pulvinate, raised, and umbonate. Edges consisted of entire, rhizoid, undulate, lobate, and filamentous. The color of bacterial colonies consisted of orange-brown, cream, light cream, dark cream, yellow, orange-yellow, white, clear white, creamy white, milky white, and cloudy white. Colony sizes consisted of small, medium, and large. Colony textures consisted of smooth, rough, dry, smooth sticky, rough dry, and dry sticky.

Proportion of Cellulolytic bacteria

This study found that bacteria produced cellulase enzyme activity, which indicates that some of them were cellulolytic bacteria. This is indicated by the presence of a clear zone formed around the bacterial colony after being given Congo red solution (Figure 2). The large clear zone formed indicates the high activity of the cellulase enzyme produced by bacteria.

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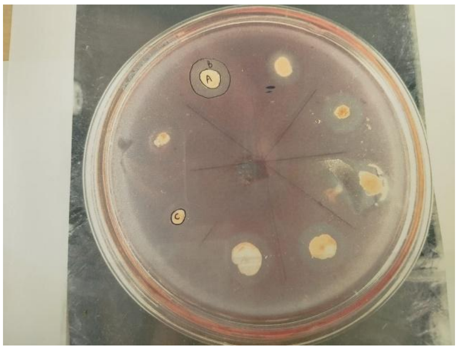

Figure 2. Cellulase Enzyme Activity Produced by Cellulolytic Bacteria. (A) Bacterial Isolates (B) Clear Zone (C) Bacterial Isolates that Do Not Produce Cellulase Enzymes.

The results of the cellulolytic activity test of bacteria showed that out of 150 bacterial isolates isolated from the digestive tract of shrimp in both the South Coast and North Coast waters, 68 isolates had cellulolytic activity. The number of bacterial isolates that had positive cellulolytic activity illustrates the proportion of cellulolytic bacteria found in the digestive tract of shrimp.

The proportion of cellulolytic bacteria from both locations with different shrimp sizes showed varying proportion values. The proportion of cellulolytic bacteria found in the digestive tract of jerbung shrimp can be seen in Table 2.

Table 2. The proportion of cellulolytic bacteria in the digestive tract of jerbung shrimp.

Location	Shrimp Size	Total number of isolates	Number of cellulolytic isolates	Proportion (%)
Pemalang	Small	25	10	40
	Medium	25	15	60
	Large	25	3	12
Cilacap	Small	25	16	64
	Medium	25	10	40
	Large	25	14	56

Distribution of cellulase enzyme activity index

The distribution of the cellulase enzyme activity index produced by bacteria in this study was obtained by dividing the diameter of the clear zone of bacteria by the diameter of the bacterial colony. The distribution value of cellulolytic bacterial activity was divided based on the type of location and size of shrimp. The cellulolytic activity index value was obtained from the highest average at an incubation time of 48 hours. The cellulolytic activity index value is presented in Figure 3.

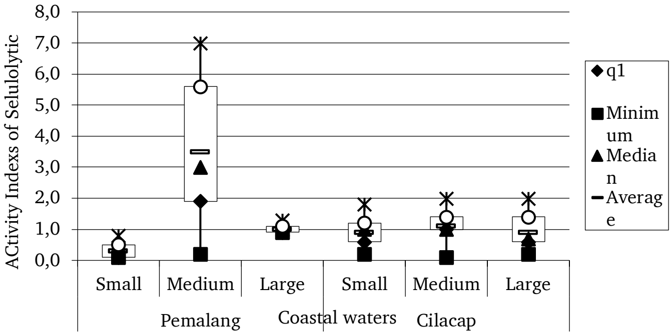

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Figure 3. Distribution of Cellulolytic Bacterial Activity Index in the digestive tract of jerbung shrimp.

Information: Quartile 1 (q1): Distribution of the lowest cellulolytic index data; Minimum: The lowest cellulolytic index value; Median: The middle value of the cellulolytic index; Mean: The average value of the cellulolytic index; Maximum: The highest cellulolytic index value; Quartile 3 (q3): Distribution of the highest cellulolytic index data; Box plot: cellulolytic index data set.

The best cellulase enzyme activity index value

There are 68 bacterial isolates from both locations that have a cellulase enzyme activity index and 11 isolates from each location that have the highest activity index with medium and strong categories. According to Choi *et al*. (2005), the cellulase activity index value is categorized into 3, namely the weak category ≤ 1 cm, the medium category 1-2 cm, and the strong category ≥ 2 cm. The best cellulase activity index value can be seen in Table 3 below.

From 22 bacterial isolates, it was narrowed down again based on the cellulolytic activity index value of>3 and as many as 8 isolates. This narrowing down was based on its potential to produce powerful cellulolytic enzymes. The highest cellulolytic bacterial isolates can be seen in Table 4.

Table 3. Bacteria with the best cellulase enzyme activity index.

Location	Best isolate	cellulolytic activity index (cm)	Category
Pemalang	GM.1	6.5	Strong
	GM.2	7.0	Strong
	GM.3	3.0	Strong
	GM.4	3.0	Strong
	GM.5	6.5	Strong
	GM.6	5.0	Strong
	GM.7	5.8	Strong
	GM.8	5.4	Strong
	GM.17	2.4	Strong
	GM.18	2.1	Strong
	GM.19	2.4	Strong
Cilacap	RS.2	1.8	Medium
	RS.4	1.5	Medium
	RS.6	1.5	Medium
	RS.11	1.4	Medium
	RM.20	1.8	Medium
	RM.21	2.0	Medium
	RM.22	1.4	Medium
	RL.14	2.0	Medium
	RL.15	1.5	Medium
	RL.16	1.7	Medium
	RL.17	1.6	Medium

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Table 4. The Highest activity of cellulolytic bacterial enzyme.

Best Isolate	Shape	Elevation	Edge	Color	Size	Texture	cellulolytic activity index (cm)	Category
GM.1	Irregular	Umbonate	Undulate	Cream	Large	Smooth	6.5	Strong
GM.2	Irregular	Pulvinate	Undulate	Cream	Medium	Smooth	7.0	Strong
GM.3	Circular	Convex	Entire	Light Cream	Medium	Smooth	3.0	Strong
GM.4	Irregular	Raised	Undulate	Cream	Medium	Smooth	3.0	Strong
GM.5	Irregular	Flat	Undulate	Cream	Medium	Smooth	6.5	Strong
GM.6	Circular	Pulvinate	Entire	Milk White	Large	Smooth	5.0	Strong
GM.7	Irregular	Convex	Undulate	Cream	Large	Smooth	5.8	Strong
GM.8	Circular	Flat	Entire	Milk White	Medium	Smooth	5.4	Strong

The abundance of bacteria found in the intestines of shrimp shows differences in size and location. According to researchers, shrimp of different sizes have dissimilar digestive tracts and food availability. Food availability in the shrimp's living environment affects the abundance of bacteria found in the digestive tract (Sharma *et al.*, 2021). According to Suwartimah *et al*. (2011) and Ye *et al*. (2020), in the period from January to March, the content of organic matter, nutrients, chlorophyll, and plankton found in the northern coastal waters tends to be higher. The high abundance of bacteria in the digestive tract of small and medium-sized shrimp indicates the role of shrimp intestinal microbiota in shrimp growth. While in large sizes, the abundance of digestive tract bacteria is less related to its function in helping the absorption of nutrients and shrimp health. Furthermore, Fan *et al*. (2019) explained that the abundance of bacteria in the shrimp digestive tract is correlated with the increase in size and weight of shrimp, whereas in adult sizes there is a decrease in the abundance of digestive tract bacteria related to the absorption of nutrients in the shrimp intestines.

The proportion of cellulolytic bacteria is calculated based on the cellulolytic activity produced by bacteria after visualization with the Congo red reagent. According to Steensma (2001), the clear zone produced by Congo red will be a reaction of Congo red and the β-1,4-glycosidic bond contained in the cellulose polymer. Where cellulose itself is hydrolyzed due to the activity of cellulolytic enzymes produced by bacteria. In our study, the highest proportion of cellulolytic bacteria was obtained in the digestive tract of shrimp isolated from the coastal waters of Cilacap. The high proportion of cellulolytic bacteria found in the shrimp digestive tract indicates the amount of cellulose-containing feed consumed. In several studies, it was explained that prawns consume the remains of organic matter, small animals, copepods, aquatic plants, phytoplankton, foraminifera, and benthos (Chong and Sasekumar, 1981). Jerbung shrimp are included in the omnivorous category, where the variety of food consumed comes from the environment (Liu *et al.*, 2016). This was proven by Kumar and Kusuma (2006) that the contents of the digestive tract of shrimp caught 66.6% were dominated by animal material and 25.7% vegetable material. Vance and Rothlisberg (2020) also stated that the results of intestinal dissection to observe the type of food consumed by gerbil shrimp some pieces are not identified. The contents of the digestive tract of gerbil shrimp depend on the availability of food in their habitat (Kumar and Kusuma, 2006).

The cellulolytic bacterial activity index values that we obtained showed

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differences in each size from the two locations. The cellulolytic activity index values from Pemalang coastal waters from small to large shrimp sizes ranged from 0.1-7.0. While in Cilacap coastal waters it ranged from 0.1-2.0. We suspect that the differences in cellulolytic activity index values are caused by several factors, including differences in strain types, shrimp sizes, and types of food consumed. Differences in cellulolytic activity index values indicate differences in the ability and types of bacterial species to hydrolyze cellulose, thus affecting the size of the clear zone formed (Dewiyanti *et al*., 2022a). According to Meryandini *et al*. (2010), different cellulolytic bacteria can produce varying cellulase enzymes, depending on the genes they have and the amount of cellulose-containing food in the environment. In addition, differences in shrimp size also affect the cellulolytic enzyme activity index value. This is explained by Gamboa-Delgado *et al*. (2003), who state that there are changes in enzymatic activity in shrimp as the size and weight of the shrimp increase.

The results of the study obtained 8 bacterial isolates that had the highest cellulolytic activity, namely isolates with codes GM.1, GM.2, GM.3, GM.4, GM.5, GM.6, GM.7, GM.8 with a range of cellulolytic indexes obtained ranging from 3.0-7.0. Bacterial isolates with the highest cellulolytic activity index were obtained from the digestive tract of jerbung shrimp isolated in the northern waters of Pemalang. This is due to the environmental conditions of the waters where jerbung shrimp live such as the availability of nutrients, water temperature, and ecological characteristics of shrimp habitat. According to Dabadé *et al*. (2015), the composition of nutrients in northern waters can support a more diverse and abundant population of cellulolytic bacteria. Where nutrient-rich environments encourage the growth of microorganisms capable of breaking down cellulose, which is very important for the digestive process in shrimp. In addition, water temperature is also a factor that affects the presence of bacteria and their ability to degrade cellulose, this is explained by Aswan *et al*. (2024) that temperatures in northern waters are cooler than in southern waters, so certain microbial communities may be better able to adapt to utilize cellulose, resulting in higher concentrations of cellulolytic bacteria. Another factor that influences the high cellulolytic index is the ecological characteristics of shrimp living habitat. According to Naresh *et al*. (2019), the characteristics of north and south coastal waters are very different. Where the characteristics of the north coast aquatic ecosystem strongly support the abundance of cellulolytic bacteria that are more diverse than the southern ecosystem due to variations in soil type, organic matter content, and microbial interactions in this ecosystem. The high cellulolytic activity index formed indicates the high ability of bacteria to produce cellulolytic enzymes (Nugraha *et al*., 2014). The high ability of bacteria to produce cellulolytic enzymes indicates their potential as probiotic agents (Ren *et al*., 2020). In our study, the highest cellulolytic bacteria were isolated from the shrimp digestive tract, which indicates that these bacteria are specifically used as probiotic agents with the advantage of being cellulase-degrading agents that function to accelerate the shrimp digestion process. This potential requires further development to determine the type of species, potential, and safety as a probiotic agent to increase shrimp growth.

CONCLUSION

The number of bacteria that have cellulolytic activity ranges from 68 isolates. A total of 22 best cellulolytic isolates have cellulolytic activity index values ranging from 1.4 to 7.0. There are also 8 isolates with the highest cellulolytic activity index, namely isolates with codes GM.1, GM.2, GM.3, GM.4, GM.5, GM.6, GM.7, GM.8 with a range of cellulolytic indexes obtained ranging from 3.0 to 7.0. The high activity index of the eight cellulolytic bacteria obtained in our study indicates their

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potential as probiotic candidates for degrading fiber in shrimp food.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript.

AUTHOR CONTRIBUTION

Ren Fitriadi: Writing - Original Draft, Conceptualization. Aninditia Sabdaningsih: Writing - Original Draft, Methodology, Review & Editing. Petrus Hary Tjahja Soedibya: Writing - Review & Editing. Slamet Budi Prayitno: Writing - Original Draft. Sarjito and Subagiyo: Writing - Review & Supervision. All authors critically revised the manuscript and approved the final version.

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