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Growth Characteristics of *Vibrio parahaemolyticus* Isolated from Lobster (*Panulirus* sp.) Under Different Temperatures, pH, and NaCl Concentrations

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Abstract

Vibrio parahaemolyticus is a bacterium found in estuaries and marine and is a pathogenic bacterium that harms human health. *Vibrio parahaemolyticus* can contaminate fishery products and potentially contaminate lobster products in North Sulawesi. This study was conducted to determine the presence of *V. parahaemolyticus* in lobsters collected in two shelters in North Sulawesi. This study also serves as a monitoring function and a means of information on the presence of *V. parahaemolyticus*. The samples used were lobsters (*Panulirus* sp.) taken from Malalayang and Tuminting shelters in North Sulawesi. Observations in this study include total bacteria, total *Vibrio*, and identified *V. parahaemolyticus*. Isolates of *V. parahaemolyticus* were characterized by their growth at different temperatures (5, 37, 43 ºC); pH (5 - 9); and concentrations of NaCl (0, 1, 3, 5%). The results show total plates for lobster meat from Malalayang 4.3×10^4 – 1.0×10^5 CFU/g, while from Tuminting shelter 1.4×10^4 – 3.9×10^4 CFU/g. The total plate on lobster gills from Malalayang is 6.2×10^4 – 1.2×10^5 CFU/g, while from Tuminting shelter 2.0×10^4 – 6.7×10^4 CFU/g. Total *Vibrio* in lobster meat from Malalayang 6.2×10^3 – 1.4×10^4 CFU/g, while from Tuminting shelter 5.2×10^3 – 7.9×10^3 CFU/g. Total *Vibrio* in lobster gills from Malalayang 8.5×10^3 – 4.6×10^4 CFU/g, while from Tuminting shelter 7.8×10^3 – 9.5×10^3 CFU/g. The Gram staining analysis obtained 96 isolate strains with Gram-negative rods, and 42 strains showed characteristics as *V. parahaemolyticus* based on the biochemical assay. *Vibrio parahaemolyticus* can grow optimally at 37°C, pH 7–8, and NaCl concentration 3–5%.

Keywords: Lobster, Malalayang, North Sulawesi, Tuminting, Vibrio parahaemolyticus

INTRODUCTION

Lobster (*Panulirus sp.)* is an economically important fishery commodity for export (Amali & Sari, 2020), which people worldwide like because of its sweet, savory, and delicious meat. The lobster (*Panulirus* sp.) commodity has the nutritional value humans need because it contains less cholesterol, calories, and essential fatty acids when compared to other types of meat (Haryono et al., 2015). This is evident from the increasing domestic market demand for lobster exports (Maskun et al., 2020).

In Indonesia, there is currently an increase in world market demand for lobsters, causing this commodity to become very popular not only limited to the domestic market but also as an export commodity, especially to countries in the Southeast Asian region, Hong Kong, Taiwan, China, and Japan (Muzayyin et al., 2019). In North Sulawesi, the status of the lobster business is still limited to marine fishing (Jamlean et al., 2018). captured lobsters are kept in temporary shelters before being marketed to maintain quality (Beard & McGregor, 2004). The lobster collecting business in North Sulawesi has been conducted since 1999 (Makasangkil et al., 2017).

As with other fishery commodities, lobster is a perishable commodity that requires proper handling at harvest time. One of the causes of damage to lobster, namely microorganisms, is a result of contamination and causes spoilage and even disease for humans who consume it (Adedeje et al., 2012). One of the aquatic microorganisms that can contaminate lobsters is *Vibrio parahaemolyticus* (Praja & Safnurbaiti, 2018).

Vibrio parahaemolyticus is a bacterium found in rivers, estuaries, ponds, and the marine. *Vibrio parahaemolyticus* are reported to contaminate fishery products such as fish, crabs, oysters, and clams (Sullivan & Neigel, 2018; Yu et al., 2013). *Vibrio parahaemolyticus* is reportedly resistant to high salinity and is a pathogenic

bacterium that harms human health (Kalburge et al., 2014). The bacterium *V. parahaemolyticus* was reported to contaminate seafood (Hara-Kudo & Kumagai, 2014). *Vibrio parahaemolyticus* has the potential to contaminate lobster products in North Sulawesi (Nakaguchi, 2013). Compared to *Vibrio cholera*, *V. parahaemolyticus* tends to contaminate lobster relatively higher, with a percentage of 10.3 % (Bauer et al., 2006).

However, the growth characteristics of *V. parahaemolyticus* still need to be fully understood. Therefore, it is necessary to isolate *V. parahaemolyticus* from contaminated lobster. Srisangthong et al. (2023) reported that characterizing *Vibrio's* growth can determine the contaminant's source so that contamination can be prevented. Furthermore, understanding the growth pattern of *Vibrio* is essential to develop effective control measures as a preventive measure for crustacea contamination (Letchumanan et al., 2014). Gámez-Bayardo et al. (2021) stated that identifying the factors contributing to *Vibrio's* growth can help improve food safety. In aquaculture, characterizing the growth of *Vibrio* can assist in developing effective disease management strategies (de Souza Valente & Wan, 2021).

Based on the description above, this study aimed to determine the contamination of *V. parahaemolyticus* in lobsters collected at two shelters in North Sulawesi. This research also contributes as a monitoring function in the development of effective preventive measures in order to improve food safety and management of disease control strategies.

MATERIALS & METHODS

Description of the study sites

The samples used were lobsters (*Panulirus* sp.) taken from the Malalayang and Tuminting shelters in North Sulawesi. Lobster sampling was carried out thrice, randomly from each shelter holding tank. The sampling period is between September – November 2022. Next, the isolation and identification of *Vibrio parahaemolyticus* from the meat and gills of lobsters (*Panulirus* sp.) was carried out at the Fisheries Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Manado.

Data Analysis

Lobster meat and gills were prepared to examine total bacteria and total *Vibrio*. Lobster meat and gills are crushed until smooth with NaCl (0.9%). Then, 1 mL was taken and put in 9 mL of NaCl (0.9%) solution.

Observations in this study include total bacteria grown on nutrient agar (NA), following to Indonesian National Standard No. 01-2332.3-2006 (BSN, 2006), total *Vibrio* grown on selective media, Thiosulfate Citrate Bile Salt Sucrose agar (TCBS Agar)(Duan & Su, 2005), Gram staining (Fabbro et al., 2010), and biochemical assays like oxidase, catalase, motility, indole, methyl red, voges-proskauer, Citrate, Carbohydrate Fermentation (glucose, maltose, lactose, sucrose) (Jones et al., 2012; Fishbein & Wentz, 1973).

Furthermore, isolates of *Vibrio parahaemolyticus* were characterized for their growth at different temperatures (5, 37, 43 ^{o}C); pH (5, 6, 7, 8, 9); and concentrations of NaCl (0, 1, 3, 5%). The growth characteristics of *V. parahaemolyticus* were carried out on nutrient broth (NB) and nutrient agar (NA) media with different temperature, pH, and NaCl concentration treatments.

RESULTS AND DISCUSSION

Samples of lobster meat and gills (*Panulirus* sp.) were analyzed for Total Plate Count (TPC) grown on NA media. The results of the Total Plate Count analysis of lobster meat and gills are presented in Table 1.

Table 1: Total Plate Count (CFU/g) in lobster (*Panulirus* sp.)

Location	Repetition	Meat	Gills
Malalayang	1	7.9×10^4	9.2×10^4
	2	4.3×10^4	6.2×10^4
	3	1.0×10^5	1.2×10^5
Tuminting	1	1.4×10^4	2.0×10^4
	2	1.9×10^4	2.1×10^4
	3	3.9×10^4	6.7×10^4

Table 1 shows that the total plate count (TPC) obtained from lobster samples at the Malalayang shelter was higher than at the Tuminting shelter, both in meat and gills. The presence of bacteria in fishery products is strongly influenced by the environment in which they live

(Kyule et al., 2022; Novoslavskij et al., 2016). Bacteria that contaminate fishery products mostly come from the surrounding environment, namely sea water, river water, and pond water which is their habitat (Atef et al., 2016).

The condition of the shelter in Malalayang is that when the lobsters arrive, they are washed first and put into the holding tanks. The holding tanks are 4 × 3 × 1 m made of cement. The seawater used in the tanks comes from Malalayang Beach, which is 100 m from the coast. Malalayang Beach, a densely populated residential area, habitually disposes of household waste into the sea (Schaduw et al., 2021). Thus, this contributes to the increasing number of contaminant bacteria in the sea and indirectly increases the number of bacteria in fish and other marine biota (Landrigan et al., 2020).

Meanwhile, in the Tuminting shelter, when the lobsters arrive, they are placed in the first holding tank for 1 - 3 hours. This initial holding aims to rinse and allow the lobsters to adapt to the conditions in the holding tanks. Next, the lobsters are moved to the next tub and separated according to type and size. The conditions holding tanks are ceramic coated with a depth of ± 80 cm with a size of 3 × 2 × 1 m equipped with an aeration system. The seawater used in the tanks came from the Tasik Ria area, which was taken 300 m from the coast.

Suppose the conditions of the two shelters correlate with the bacterial TPC analysis results. In that case, it is reasonable that the bacterial TPC analysis of the Tuminting shelter is lower than that of the Malalayang shelter. This result is because the quality of the Tuminting shelter is relatively better than the Malalayang shelter. From the TPC results on lobster meat and gill samples, it can be seen that in the gills, the number of bacteria is relatively higher. This result is because the gills are the first organ to receive food, in addition to being a respiratory device that plays a role in filtering particles contained in water, including organic matter, sand, and microorganisms (Kumari et al., 2009). According to SNI No. 7388:2009, the quality standard for total plate count for fresh fishery products (including mollusks, crustaceans, and echinoderms) is 5×10^5 CFU/g (BSN, 2009).

Furthermore, to grow *Vibrio* bacteria, the suspensions of lobster meat and gill samples were grown on TCBS (Thiosulfate Citrate Bile Salt Sucrose) selective media. TCBS agar is the standard medium commonly used for the selective isolation and further identification of *Vibrio* spp. (Baker et al., 2018). TCBS agar medium is a selective medium used to grow *Vibrio* colonies. TCBS medium contains sucrose as the primary carbon source and electrolytes ($MgCl_2$-$6H_2O$ and KCl), which stimulate bacterial growth (Cerdà-Cuéllar et al., 2000). *Vibrio* colonies in TCBS agar medium produce a yellow color with colonies that are large, smooth, puck, transparent, thin edges, surrounded by a yellow zone, and some colonies that form have a green color (Figure 1) (Hara-Kudo et al., 2001). Total colonies of *Vibrio* on lobster from Malalayang and Tuminting Shelters can be seen in Table 2.

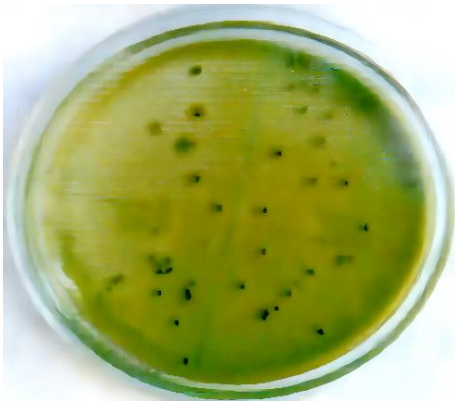


Figure 1. *Vibrio* colonies on TCBS agar media

Table 2. Total *Vibrio* count (TVC/g) on lobster (*Panulirus* sp.)

Location	Repetition	Meat	Gills
Malalayang	1	$1{,}2 \times 10^4$	$1{,}8 \times 10^4$
	2	$1{,}4 \times 10^4$	$4{,}6 \times 10^4$
	3	$6{,}2 \times 10^3$	$8{,}5 \times 10^3$
Tuminting	1	$5{,}2 \times 10^3$	$9{,}5 \times 10^3$
	2	$7{,}1 \times 10^3$	$7{,}8 \times 10^3$
	3	$7{,}9 \times 10^3$	$8{,}9 \times 10^3$

Table 2 shows that the total *Vibrio* in the gills and meat from the Malalayang shelter was higher than the total *Vibrio* in the gills and meat from the Tuminting shelter. Several factors, including the nature of omnivorous lobsters, scavengers, and same-sex eaters, can cause the high *Vibrio* content in lobsters. In nature, lobsters

eat various living and dead organisms, especially benthic animals from the Mollusca group (gastropods and bivalves) and Echinodermata (sea urchins, sea cucumbers, starfish) (Serratore et al., 2021). The presence of *Vibrio* in lobsters can also be caused by the polluted waters where they are caught (Raissy et al., 2012). The number of *Vibrio* will continue to increase if the sanitation and hygiene of seawater used in the holding tanks are ignored (Brumfield et al., 2023).

The growing colonies in TCBS agar were isolated to pure culture and analyzed by Gram staining to obtain Gram-negative bacterial isolates. *Vibrio parahaemolyticus* is Gram-negative-rod (Kalburge et al., 2014), with a colony diameter of 2-3 mm, smooth and flat (Kourany, 1983). The Gram staining analysis obtained 96 strains of isolates with Gram-negative rods. From 96 strains of bacterial isolates, suspected *Vibrio* was then continued with biochemical testing (oxidase, catalase, motility, indole, methyl red, Voges-Proskauer, citrate, carbohydrate fermentation). The results of biochemical testing of 96 strains of bacterial isolates are presented in Table 3 and Figure 2.

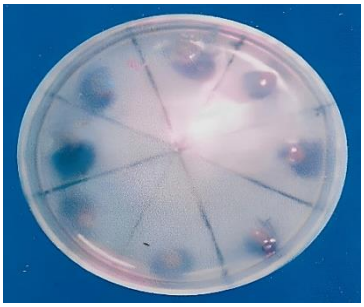
Table 3. Biochemical assay of 96 strains of Gram-negative-rod isolate (suspected *Vibrio*)

Isolate	Oxidase	Catalase	Motility	Indole	Methyl Red	Voges-Proskauer	Citrate	Carbohydrate Fermentation			
								Glu-cose	Mal-tose	Lac-tose	Suc-rose
I.MD1	+	+	+	+	+	-	-	A	A	-	-
I.MD2	+	+	+	+	+	-	-	A	A	-	-
I.MD3	+	+	+	+	+	-	-	A	A	-	-
I.MD4	+	-	-	+	+	-	-	A/G	A	A	A/G
I.MD5	+	+	-	+	-	-	-	A	A	A/G	A/G
I.MD6	+	+	+	+	+	-	-	A	A	-	-
I.MD7	+	+	+	+	+	-	-	A	A	-	-
I.MD8	+	+	+	+	-	-	-	A	A	A	A
I.MI1	+	+	+	+	+	-	-	A	A	A	-
I.MI2	+	+	+	+	+	-	-	A	A	-	-
I.MI3	+	+	+	+	+	-	-	A	A	-	-
I.MI4	+	+	+	+	+	-	-	A	A	-	-
I.MI5	+	+	+	+	+	-	-	A	A	A	-
I.MI6	+	+	+	+	+	-	-	A	A	A	-
I.MI7	+	+	+	+	+	-	-	A	A	-	-
I.MI8	+	+	+	+	+	-	-	A	A	-	-
I.TD1	+	+	+	+	+	-	-	A	A	A	A
I.TD2	+	+	+	+	+	-	-	A	A	A	A/G
I.TD3	+	+	-	+	-	-	-	A	A/G	A	A
I.TD4	+	+	+	+	+	-	-	A	A	-	-
I.TD5	+	+	+	+	+	-	-	A	A	-	-
I.TD6	+	+	-	+	-	-	-	A	A	A	A/G
I.TD7	+	+	+	+	-	-	-	A	A	-	-
I.TD8	+	+	+	+	+	-	-	A	A	A	-
I.TI1	+	+	+	+	+	-	-	A	A	-	-
I.TI2	+	+	+	+	+	-	-	A	A	-	-
I.TI3	+	+	+	+	+	+	-	A	A	-	A
I.TI4	+	+	+	+	+	-	-	A	A	-	-
I.TI5	+	+	+	+	-	-	-	A	A	-	-
I.TI6	+	+	+	+	+	-	-	A	A	A	-
I.TI7	+	+	+	+	+	-	+	A	A	-	A
I.TI8	+	+	+	+	+	-	-	A	A	-	-
II.MD1	+	+	+	+	+	-	-	A	A	-	-
II.MD2	+	+	+	+	+	-	-	A	A	-	-
II.MD3	+	+	+	+	-	-	-	A	A	-	-
II.MD4	+	+	+	+	-	-	-	A	A	-	-
II.MD5	+	+	+	+	+	-	-	A	A	-	-
II.MD6	+	+	+	+	+	-	-	A	A	-	-

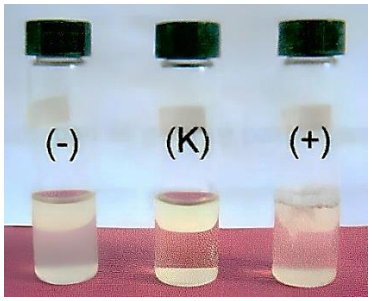
Isolate	Oxidase	Catalase	Motility	Indole	Methyl Red	Voges-Proskauer	Citrate	Carbohydrate Fermentation			
								Glu-cose	Mal-tose	Lac-tose	Suc-rose
II.MD7	+	+	+	+	+	-	+	A	A	-	-
II.MD8	+	+	+	+	+	-	+	A	A	-	-
II.MI1	+	+	+	+	+	-	-	A	A	-	-
II.MI2	+	+	+	+	+	-	+	A	A	-	-
II.MI3	+	+	+	+	+	-	+	A	A	-	-
II.MI4	+	+	+	+	+	-	-	A	A	-	-
II.MI5	+	+	+	+	+	-	-	A	A	-	-
II.MI6	+	+	+	+	-	-	-	A	A		-
II.MI7	+	+	+	+	+	-	-	A	A	-	-
II.MI8	+	+	+	+	+	-	-	A	A	-	-
II.TD1	+	+	+	+	-	-	-	A	A	-	-
II.TD2	+	+	+	+	-	-	-	A	A	-	-
II.TD3	+	+	+	+	+	-	-	A	A	-	-
II.TD4	+	+	+	+	+	-	+	A	-	-	A/G
II.TD5	+	+	+	+	+	-	-	A	-	A	-
II.TD6	+	+	+	+	+	-	-	A	A	-	-
II.TD7	+	+	-	+	+	-	-	A	A	-	A
II.TD8	+	+	+	+	-	-	-	A/G	A/G	-	A
II.TI1	+	+	+	+	+	+	-	A	A	A	A
II.TI2	+	+	+	+	-	-	-	A	A	A	A
II.TI3	+	+	+	+	+	-	-	A	A	A	A
II.TI4	+	+	+	+	+	+	-	A	-	A	-
II.TI5	+	+	+	+	-	-	+	A	A	-	-
II.TI6	+	+	+	+	+	-	-	A	A	-	-
II.TI7	+	+	+	+	+	-	-	A	A	-	-
II.TI8	+	+	+	+	+	-	-	A	A	-	-
III.MD1	+	+	+	+	+	-	-	A	A	-	-
III.MD2	+	+	-	+	-	-	-	A	A	A	A
III.MD3	+	+	+	+	-	-	-	A	A	-	-
III.MD4	+	+	+	-	-	+	+	A	A	A	A/G
III.MD5	+	+	-	+	-	-	-	A	A	A	A
III.MD6	+	+	+	+	+	-	-	A	A	-	-
III.MD7	+	+	+	+	+	-	-	A	A	-	-
III.MD8	+	+	-	+	-	-	-	A	A	-	A/G
III.MI1	+	+	+	+	+	-	-	A	A	-	-
III.MI2	+	+	+	+	+	-	-	A	A	-	-
III.MI3	+	+	+	+	-	-	-	A	-	A	A/G
III.MI4	+	+	+	+	+	-	-	A	-	-	A/G
III.MI5	+	+		+	+	+	-	A	A/G	A/G	A/G
III.MI6	+	+	+	+	+	-	-	A	A	-	-
III.MI7	+	+	-	+	-	+	-	A	A	A	A/G
III.MI8	+	+	-	+	-	-	-	A	A	A	A/G
III.TD1	+	+	+	+	+	-	-	A	A	-	-
III.TD2	+	+	+	+	+	-	-	A/G	A/G	A/G	A/G
III.MTD3	+	+	+	+	+	-	-	A	A	-	-
III.TD4	+	+	+	+	-	+	-	A	A	A	-
III.TD5	+	+	+	+	-	+	-	A	A	A	A
III.TD6	+	+	+	+	+	-	-	A	A	-	-
III.TD7	+	+	-	+	-	+	-	A	-	A	-
III.TD8	+	+	+	+	-	-	-	-	-	-	-
III.TI1	+	+	+	+	+	-	-	A	A	-	-
III.TI2	+	+	+	+	+	+	-	A	A	-	-
III.TI3	+	+	+	+	-	+	-	A	A	A	A
III.TI4	+	+	-	-	-	+	-	A	A	A	A
III.TI5	+	+	+	+	+	-	-	A	A	-	-
III.TI6	+	+	+	+	+	-	-	A	A	-	-

Isolate	Oxidase	Catalase	Motility	Indole	Methyl Red	Voges-Proskauer	Citrate	Carbohydrate Fermentation			
								Glu-cose	Mal-tose	Lac-tose	Suc-rose
III.TI7	+	+	-	+	-	-	-	A	-	A	A
III.TI8	+	+	+	+	-	-	-	A	-	-	-

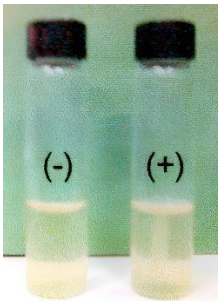
= Identified as *Vibrio parahaemolyticus*
I, II, III = Sampling n[th]
M, T = Malalayang, Tuminting
D, I = Meat, Gills
1-8 = Bacterial colonies n[th]
A, G = Acid, Gas



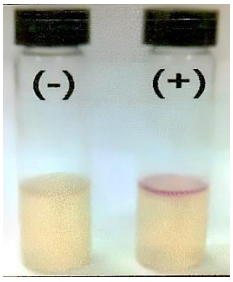
Oxidase
(+)=pink colonies



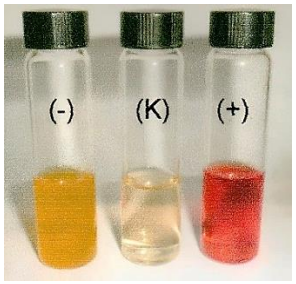
Catalase
(+)= positive; (K)=control;
(–)=negative



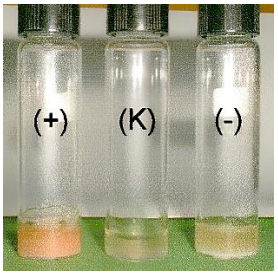
Motility
(+)= positive; (–)=negative



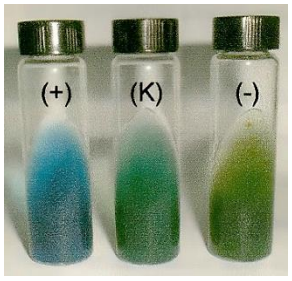
Indole
(+)= positive; (–)=Negative



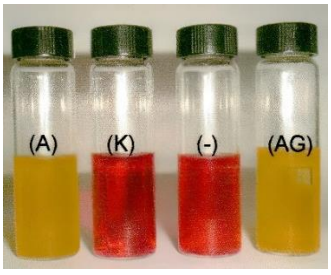
Methyl Red
(+)= positive; (K)=control;
(–)=negative



Voges-Proskauer
(+)= positive; (K)=control;
(–)=negative



Citrate
(+)= positive; (K)=control; (–)=negative



Carbohydrate Fermentation
(A)= acid; (K)=control; (–)=negative; AG=acid and gas

Figure 2. Biochemical assay of Gram-negative-rod isolate (suspected *Vibrio*)

Based on characteristics, *Vibrio parahaemolyticus* was reported to give a positive response to oxidase, catalase, motility, indole, and methyl red assays; and a negative reaction to the Voges-Proskauer and citrate assays (Jones et al., 2012; Nickelson & Vanderzant, 1971). In the

carbohydrate fermentation assay, *V. parahaemolyticus* was reported to be able to ferment glucose and maltose by forming acid without producing gas and did not ferment lactose and sucrose (Bryant et al., 1986; Lim, 1976). Table 3 shows that of the 96 strains of bacterial isolates suspected to be *Vibrio*, 42 strains showed characteristics as *V. parahaemolyticus* based on the literature.

Vibrio is generally oxidase and catalase positive, reduces nitrate to nitrite, is halophilic, requires NaCl for growth, and cannot ferment lactose (Brennan-Krohn et al., 2016). This positive oxidase property is a characteristic of *Vibrio* that distinguishes it from Enterobacteriaceae (Vila et al., 1992). In the catalase assay, *V. parahaemolyticus* isolates show catalase positive because it has a catalase enzyme that can degrade H_2O_2 into H_2O and O_2 (Ichise et al., 1999). Catalase enzyme is an antioxidant and protects *V. parahaemolyticus* from oxidative stress (Lin et al., 2015). In the motility assay, isolate *V. parahaemolyticus* showed positive motility because it is a motile bacterium with polar flagella (Fishbein & Wentz, 1973). *Vibrio parahaemolyticus* is reported to have two different types of flagella with different functions for swimming and swarming, as well as the ability to produce capsules. These two flagella assist *V. parahaemolyticus* strains in surviving in the environment and colonizing human hosts (Letchumanan et al., 2014).

In the indole assay, *V. parahaemolyticus* strain showed a positive reaction because it has tryptophan deaminase enzyme (Khouadja et al., 2013). The indole test is done to determine the ability of bacteria to degrade the amino acid tryptophan (Lee & Lee, 2010). In the methyl red assay, *V. parahaemolyticus* gave a positive reaction; this indicates that *V. parahaemolyticus* can oxidize glucose to produce several organic acids, such as lactic acid, acetic acid, and formic acid (Lim, 1976). The methyl red assay contradicts the results of the Voges-Proskauer assay, which gives a negative response to the isolate *V. parahaemolyticus*. The positive glucose fermentation assay of the isolate *V. parahaemolyticus* aligns with the methyl red assay, where *V. parahaemolyticus* can break down glucose to form acid without producing gas. *Vibrio parahaemolyticus* is reported to break down glucose anaerobically via the Embden-Meyerhof pathway and produce formic acid, lactic acid, acetic acid, succinic acid, ethanol, and pyruvate (Percival & Williams, 2014). The maltose fermentation assay of the isolate *V. parahaemolyticus* is positive because *Vibrio parahaemolyticus* has an enzyme that can break down maltose into simpler molecules that can be used as an energy source (Letchumanan et al., 2015; Jones et al., 2012). In contrast, Table 3 shows that *V. parahaemolyticus* isolates cannot ferment lactose and sucrose. Lactose is a disaccharide of glucose and galactose, while sucrose is a disaccharide of glucose and fructose. *Vibrio parahaemolyticus* lacks the enzymes to break down lactose and sucrose into their component sugars (Regmi & Boyd, 2019).

The negative response of *V. parahaemolyticus* isolates in the Voges-Proskauer assay is because *V. parahaemolyticus* produces acid. In contrast, if the positive response is in the Voges-Proskauer assay, the bacteria have a neutral end product from glucose fermentation (Jones et al., 2012; Broberg et al., 2011). The ability of *V. parahaemolyticus* to oxidize glucose is closely correlated with its pathogenicity (Fabbro et al., 2010). Strains of *V. parahaemolyticus* exhibit an alkaline top and acidic bottom and do not produce H_2S (Kim et al., 1999). The negative response of *V. parahaemolyticus* isolates in the citrate assay indicates that *V. parahaemolyticus* cannot utilize citrate as the sole carbon source. The citrate test is performed to observe whether bacteria can utilize citrate as the sole carbon source (Brocker et al., 2009). *Vibrio parahaemolyticus* gave negative results in the citrate assay, presumably because *V. parahaemolyticus* lacks the citrate permease enzyme needed to absorb citrate into cells. Lack of citrate permease enzyme, causing *V. parahaemolyticus* cannot to utilize citrate as the only carbon source, resulting in a negative citrate test (Kostiuk et al., 2023; Letchumanan et al., 2014).

Furthermore, two isolates of *V. parahaemolyticus* (I.TD4 and III.MI6) were used to represent 42 strains of *V. parahaemolyticus* in growth characterization at different temperatures,

pH, and NaCl concentrations (Figure 3). Figure 3 shows that at 5°C, the growth index of *V. parahaemolyticus* was still low and grew well at 37°C, while at 43°C, there was a decrease in the growth index.

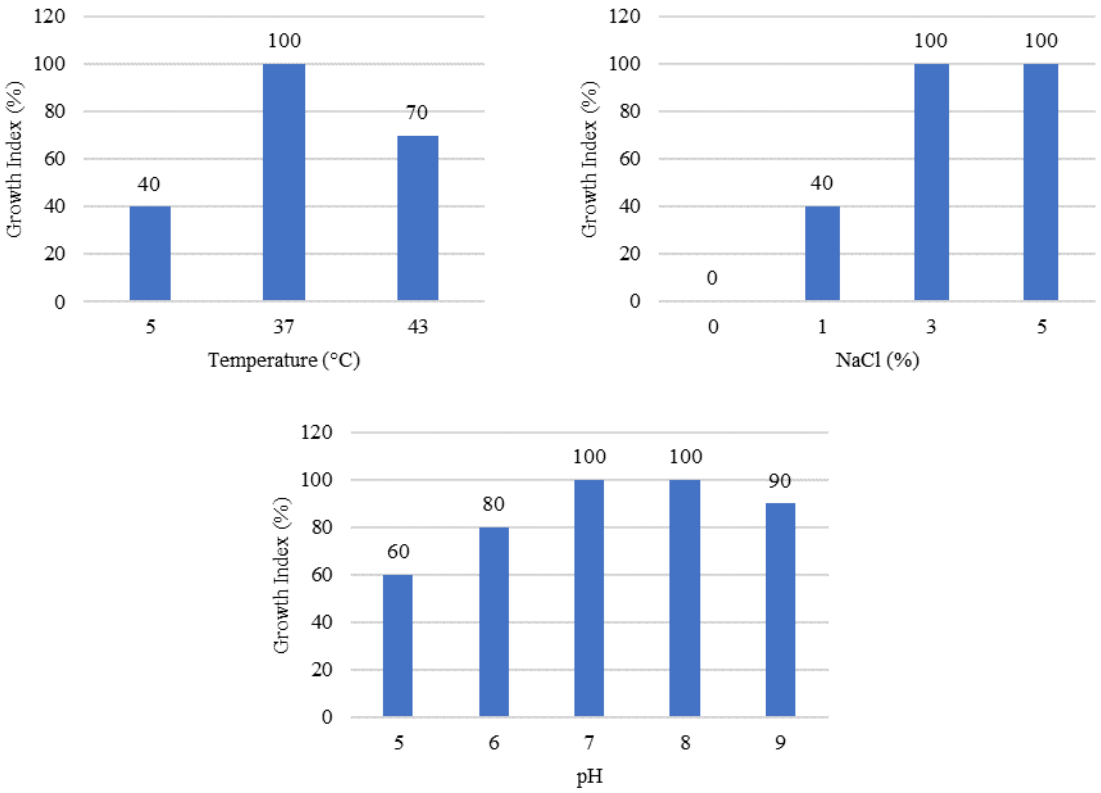


Figure 3. Growth index of *Vibrio parahaemolyticus* at different temperatures, pH, and NaCl concentrations

Figure 3 also shows that *V. parahaemolyticus* grows well at pH 7 and 8, and there is a decrease in the growth index at pH 5, 6, and 9. Previous research reported that *V. parahaemolyticus* could grow well at pH 7.5-8.5 (Chimalapat et al., 2020), while at low pH (0.9-1.5), it cannot grow (Wang et al., 2020). The results of growth characterization also show that *V. parahaemolyticus* can grow well at 3 and 5% NaCl concentration, while at 0 and 1% NaCl concentration, there is a decrease in the growth index. Previous research reported the ability to grow *Vibrio* gradually decreased with increasing NaCl concentration (>6%) and decreased growth at NaCl concentration (<1.0%) (Hu et al., 2022). Another study reported that *V. parahaemolyticus* reached the maximum growth rate in the optimum environment with conditions of 37°C and 3% salinity (Liu et al., 2016). At 0.5% NaCl concentration, the fitness and virulence of *V. parahaemolyticus* were significantly reduced (Zhang et al., 2023). Overall, different NaCl concentrations can affect *Vibrio parahaemolyticus* growth, with low salinity negatively impacting growth indices. However, the specific effect of NaCl concentration on growth rate can depend on other environmental factors such as temperature and pH.

CONCLUSION

These research findings show that the sanitation and hygiene conditions of seawater used in lobster (*Panulirus* sp.) shelters affected the total bacteria and *Vibrio* in meat and gills. The results

also found that 96 strains of Gram-negative-rod bacteria were suspected to be *Vibrio*, and about 43.75% (42 strains) were identified as *Vibrio parahaemolyticus*. *Vibrio parahaemolyticus* can grow optimally at 37°C, pH 7-8, and NaCl concentration 3-5%. Knowing the growth characteristics of *V. parahaemolyticus* concerning temperature, pH, and NaCl can be utilized in developing disease control strategies in lobster aquaculture and enable food processors to implement appropriate measures to prevent or reduce the growth of *V. parahaemolyticus* in food products.

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