NUSANTARA BIOSCIENCE
Vol. 14, No. 2, pp. 340-346
November 2022

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n140216

Detection of *Vibrio* sp. on vaname shrimp (*Litopanaeus vannamei*) cultivation pond in Gorontalo, Indonesia

PENI ULFIANI, ANI M. HASAN, YULIANA RETNOWATI♥
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo. Jl. Prof. BJ Habibie, Bone Bolango 96119, Gorontalo, Indonesia. Tel. +62-435-821125, ♥email: yuliana.retnowati@ung.ac.id

Manuscript received: 10 August 2022. Revision accepted: 30 November 2022.

Abstract. *Ulfiani P, Hasan AM, Retnowati Y. 2022. Detection of Vibrio sp. on vaname shrimp (*Litopanaeus vannamei*) cultivation pond in Gorontalo, Indonesia. Nusantara Bioscience 14: 340-346. Vibrio* is a pathogenic bacteria that can attack cultured shrimp. Therefore, it is necessary to increase the intensity of water changes and efforts to control water quality to maintain dissolved oxygen levels and manage organic matter content in ponds to prevent the increase of pathogenic bacteria, especially *Vibrio*. In addition, water physicochemical factors can influence the presence and population of *Vibrio* bacteria in ponds. This study aims to determine the presence of *Vibrio* sp. in vaname shrimp culture ponds in Gorontalo, Indonesia. Descriptive quantitative analysis was used to analyze the data collected from this study. Shrimp pond water samples were obtained from shrimp ponds in Molosipat Village, West Popayato Sub-district, Pohuwato District, Gorontalo, Indonesia, using a purposive sampling technique with an interval of three days from the first water change to the last before the water change in one period (approximately 10 days). The water samples were subjected to physicochemical analysis, including temperature, pH, salinity, ammonia, nitrate, and dissolved oxygen. The results showed that there were *Vibrio* bacteria in the Thiosulfate Citrate BileSalt Sucrose Agar (TCBSA) medium with a population that tends to increase in the range of 1.0×10^3 - 2.7×10^4 CFU/mL. The isolation results showed that two isolates were named ISL1 and ISL2, with the colony morphology characters of ISL1 isolates being green and ISL2 isolates being yellow. The results of microscopic observations of the two isolates showed comma-shaped and gram-negative cells. Molecular identification using the 16S rRNA gene and reconstruction of the phylogenetic tree using the Neighbor-Joining algorithm showed that ISL1 isolate is very closely related to *Vibrio parahaemolyticus* strain 11-1 (relationship level 99%) and ISL2 is closely related to *V. parahaemolyticus* strain B2-1 (relationship level 86%).

Keywords: *Litopanaeus vannamei*, pathogenic bacteria, shrimp pond, *Vibrio* sp., water physicochemistry

INTRODUCTION

Vaname shrimp is a cultured shrimp with high economic and nutritional value with the composition of essential amino acids, fats, macrominerals, and microminerals (Mika et al. 2013). Some of the advantages of vaname shrimp are having the ability to regulate the balance of fluid and pond water concentrations (osmoregulation) (Haliman and Adijaya 2005), high survival rate, resistance to high density, disease resistance, and low feed conversion (Supono 2018). Vaname shrimp growth is influenced by abiotic factors closely related to water quality and biotic, especially pathogenic microorganisms in the pond aquatic ecosystem. According to Supono (2018), various biota activities in the pond ecosystem can decrease water quality. Water quality conditions are related to the physicochemical conditions of the habitat and affect the growth of microorganisms. The activity of aquatic biota metabolites in using organic materials can release ammonium and nitrate as waste products of metabolism and have toxic properties for cultured shrimp. Vaname shrimp growth is also influenced by biotic factors, especially pathogenic microorganisms in the pond aquatic ecosystem. Wahyuni et al. (2017) reported that microorganisms often found in pond ecosystems are *Escherichia coli, Salmonella* sp., *Vibrio* sp., *Aeromonas* sp., and *Staphylococcus* sp.

Bacteria are the most common cause of disease in shrimp culture, especially *Vibrio* sp. Bacteria *Vibrio* sp. are pathogenic bacteria that can survive in environments with high salinity. *Vibrio* bacteria live in seawater and fresh water, are associated with marine and freshwater animals, are in normal flora and can increase with environmental conditions. Several types of bacteria of the *Vibrio* genus that cause *Vibrio*sis in shrimp include *Vibrio harveyi, v. Parahaemolyticus, v. Alginolyticus, v. Anguillarum, v. Vulnificus,* and *v. Splendidus* (Jayasree et al. 2006). *Vibrio*sis is the most common disease that attacks shrimp from the hatchery period, which causes huge losses due to death (Fatmala et al. 2019; Satomayor et al. 2019). In addition, *Vibrio* bacteria can infect shrimp, commonly called firefly disease or fluorescent disease. Transmission of *Vibrio* sp. bacteria occurs horizontally through water or contact between individuals with a high transmission rate (Zhou et al. 2012). The diseases caused by *Vibrio* could also infect humans and aquatic animals. Furthermore, *Vibrio* bacteria can also cause economic losses in the aquaculture industry.

Vibrio bacteria in an area are influenced by environmental factors, such as temperature, pH, water activity, oxidizing-reduction ability, food structure, dissolved oxygen, and total organic matter available in the host (Ilmiah et al. 2012). Compared to other microorganisms, the presence of *Vibrio* must be noticed because *Vibrio* has a wider niche and can survive in an environment with or without oxygen, which is facultatively anaerobic and pathogenic to shrimp. Soto-Rodriguez et al. (2015) reported that in 2013, atypical shrimp deaths occurred in ponds in northwest Mexico that occurred mainly in the first days after stocking. Pantjara and Hidayah (2014) stated that the availability of organic matter caused the high *Vibrio* bacteria in ponds, which is becoming good nutrition for bacterial growth.

The development and increase of microorganisms are quite rapid because these microorganisms are of their ability to utilize carbon from the available waste. Shen and Bartha (1996) in Pantjara and Hidayah (2014) stated that bacteria using organic waste produce carbon dioxide. While in a different condition or place, it will affect the diversity of *Vibrio*; besides that, the influencing factor is the biotic factor, namely competition for food in an area and the interaction of *Vibrio* with other species. Sarjito et al. (2015) reported that shrimp infected with *Vibrio* bacteria experienced clinical symptoms such as reddish uropods, pleopods, and antennal scales and experienced necrosis, brown hepatopancreas, and melanosis on the abdomen. Jayasree et al. (2006) also stated that the symptoms of *Vibrio*sis are characterized by flakes on the tail and swimming legs, empty shrimp carapace, and movement weakness. This study aims to determine the presence of *Vibrio* sp. bacteria in Vaname shrimp aquaculture ponds in Gorontalo.

MATERIALS AND METHODS

Study area

Samples of shrimp pond water were obtained from shrimp ponds in Molosipat Village, West Popayato Sub-district, Pohuwato District, Gorontalo, Indonesia, and bacterial isolation was carried out at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo, Indonesia.

Tools and materials

The tools used are sterile sample bottles, Erlenmeyer, petri dishes, hotplate (IKA® C-MAG HP 7), autoclave (Gummy Sturdy SA-300VL), centrifuge (Hettich EBA 8S), analytical balance (Pioneer™), beaker, measuring cup, oven (Memmert), incubator (Memmert), laminary air flow (Envair), vortex (Jelo Tech), thermometer, refractometer, thermocycler (Bio-Rad), electrophoresis (NanBei), spectrophotometer (Thermo Scientific Genesys 10 S UV), pH meter (Pen type pH-009) coolbox. The materials used are Vaname shrimp pond water, Thiosulfate Citrate BileSalt Sucrose Agar (TCBSA) media, Sodium Agar (NA) media, 0.9% NaCl, aquadest, reagents nitrate, and reagents ammonia, agarose, primer 27F, primer 1492R, dd H2O, MyTaq Red Mix, DNA Templates.

Procedure

Physicochemical analysis of water

Physicochemical measurements of water in ponds include pH, salinity, and temperature. In addition, the determination of ammonia, nitrate, and dissolved oxygen levels was carried out in the laboratory by spectrophotometric methods using a spectrophotometer.

Population counting and isolation of bacteria and Vibrio sp.

One (1) mL of water sample was put into 9 mL of 0.9% NaCl, and then serial dilutions were carried out from 10-1 to 10-3. *Vibrio* sp., which are marked in yellow and green on TCBSA medium. Colonies that grew separately were taken using a loop and transferred to TCBSA medium on a streak plate. The Petri dish was incubated at 30 °C for 24 hours. Total bacteria and total *Vibrio* sp. calculated using the total plate number formula based on SNI 2332.3:2015, namely:

$$N = \frac{\Sigma C}{(1xn1) + (0{,}1xn2)(d)}$$

Where:
N : Total colonies on product
C : The number of colonies in each plate counted
n1 : Number of cups in the first calculated dilution
n2 : Number of cups in the second calculated dilution
d : the first calculated dilution

The isolated bacterial isolates that grew separately were purified, and the colonies were taken using an ossicle and then grown on an inclined TCBSA medium. Each dilution was taken as much as 1 mL of sample and inoculated into TCBSA medium by pouring the plate. Petri dishes were incubated upside down at 30°C ± 1°C for 24 hours ± 2 hours. Yellow and green colonies characterize *Vibrio* bacterial colonies that grow.

Bacterial molecular identification

Bacteria were identified based on their morphological and molecular characteristics. Macroscopic morphological characteristics include colony color, colony shape, colony margin, and colony surface, while microscopic characteristics include cell shape and cell response to color. Molecular characteristics underwent several stages: DNA extraction, 16S rRNA gene amplification, visualization on agarose gel electrophoresis, 16S rRNA gene sequencing, and relationship analysis through phylogenetic tree reconstruction.

DNA extraction

DNA extraction genome analysis was performed using the Quick-DNA bacterial miniprep kit (Zymo Research, D6005). The DNA extraction results were quantitatively purified using a spectrophotometer (NanoDrop) with a 260/280 nm wavelength.

Amplification PCR

Amplification PCR using MyTaq HS Red Mix (Bioline, BIO-2504B) (Components: dd H2O, MyTaq Red Mix 2x, primer 27F (27F: 5' -AGAGTTTGATCMTGGCTCAG-3'), Primer 1492R: (5' - GGTTACCTTGTTACGACTT- 3') and DNA template, spin down with the use of following Table 1.

Electrophoresis

As much as 0.75 grams of agarose was weighed and added to 50 mL of TBE IX, then heated until all the agarose was completely dissolved. Then the agarose is printed on the Electrophoresis Tray mold until it solidifies. The PCR product was put into the well and connected to a power source with a voltage of 100 Volts, 400 mA, for 60 minutes. DNA bands were visualized under UV.

Sequencing

The sequencing of the 16S rRNA gene was carried out to determine the nucleotide sequence in the DNA fragments detected from the visualization results of DNA amplified in the PCR process using an auto-sequencing machine. In addition, data sequences of the 16S rRNA gene were performed by contig, BLAST, and alignment using Cluster W.

Reconstruct

The phylogenetic tree was conducted and prepared using the distance-based method, namely Neighbor-Joining Tree with 1000 × bootstrap in MEGA 11 software.

Data analysis

The research data were analyzed descriptively, namely describing the type of *Vibrio* sp. based on phenetic and phylogenetic morphological characters in the form of a systematic description and data presented in tables and figures.

RESULTS AND DISCUSSION

Population calculation and bacterial isolation of *Vibrio* sp.

Water quality conditions in Vaname shrimp ponds based on physicochemical analysis, including temperature, pH, salinity, ammonia, nitrate, and dissolved oxygen, were observed at intervals of three days, namely observations I, II, and III. Although the analysis results showed that temperature, pH, and salinity parameters tended to be the same in the three observations, the ammonia parameter increased in the second observation and decreased in the third observation. Meanwhile, nitrate and dissolved oxygen tend to decrease in the three observations. That is directly proportional to the total bacteria and *Vibrio*, which experienced an increase in population, and the highest population was observed in observation III (Table 2).

Water quality conditions are closely related to physicochemical conditions and become a limiting factor for the growth of microorganisms in the pond ecosystem. Therefore, microbiological parameters were used as water quality indicators for groups of pathogenic bacteria, which is the most common cause of disease in shrimp culture, especially *Vibrio* sp. The results of the ALT calculation show that the total common bacteria in ponds increased during the observation, with the highest total bacteria 4.1×10^4 CFU/mL. Still, this result was lower compared to the results of the Idami and Nasution (2020) study, where the total common bacteria in shrimp ponds reached 2.0×10^5 CFU/mL, while the total *Vibrio* in ponds tended to increase during the observation with the highest total *Vibrio* reaching 2.3×10^4 CFU/mL. According to Ganesh et al. (2010), the maximum limit of aquatic *Vibrio* is 10^3 CFU/mL.

Two isolates with different colony morphology were obtained in isolation of *Vibrio* sp. on TCBSA selective medium. Each isolate was then named ISL1 and ISL2. The ISL1 isolates showed green colony morphology, rounded shape, wavy edges, and raised colony surfaces, while ISL2 isolates showed yellow colony morphology, round shape, wavy edges, and embossed colony surfaces (Figure 1.A-B).

Based on microscopic observations to determine the shape of the cells and their response to gram staining, it was found that ISL1 and ISL2 showed comma-shaped and gram-negative cells (Figure 2.A-B). In addition, the morphological characteristics of ISL1 and ISL2 isolates showed *Vibrio* characters, namely gram-negative bacteria with bent rods (commas) (Idami and Nasution 2020).

ISL1 and ISL2 isolates were characterized by molecular characterization based on the 16S rRNA gene. The results of the extraction of the genomic DNA of the two isolates were quantitatively purified using a spectrophotometer with a wavelength of 260/280 nm and obtained DNA purity of 1.93 and 1.91, respectively. These results indicated that the extraction results reached purity and met the requirements to use at the gene amplification stage. Amplifying the 16S rRNA gene using primers 27F and 1492R and 35 cycles resulted in a DNA band length of 1,500 bp (Figure 3). The amplification product was sequenced based on the be-directional method using primers 27F and 1492R, and the results obtained were sequences at lengths of 1423bp and 1419bp.

Table 1. PCR machine protocol

Step	Temperature °C	Duration	Cycles
Initial denaturation	95	3 min	1
Denaturation	95	15 sec	35
Annealing	52	30 sec	
Extension	72	45 sec	
Final Extension	72	3 min	1
Hold	4	∞	1

Table 2. Physicochemical and total bacterial analysis of pond water

Parameters	Observation		
	I	II	III
Temperature (°C)	31.5	32.5	32
pH	8	8	8.1
Salinity (ppt)	21	22	2
Ammonia (mg/L)	0.31	0.97	0.40
Nitrates (mg/L)	0.5	0	0
Dissolved Oxygen (mg/L)	35.2	31.8	21.1
Total Bacteri (CFU/mL)	2.0×10^4	3.7×10^4	4.1×10^4
Total *Vibrio* (CFU/mL)	1.0×10^3	9.7×10^3	2.3×10^4

Note: I: First 3 days (3rd day), II: Second 3 days (6th day), III: Third 3rd day (9th day)

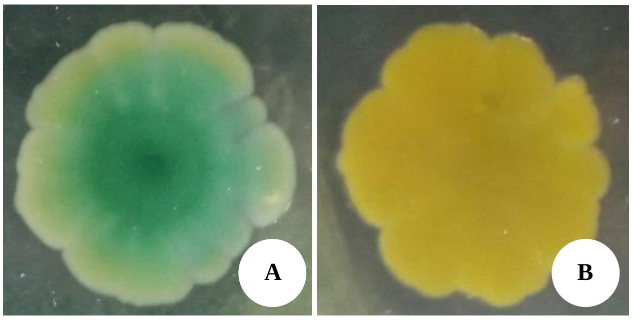

Figure 1. Bacterial isolates of *Vibrio* sp. on TCBS agar medium: A. ISL1, B. ISL2

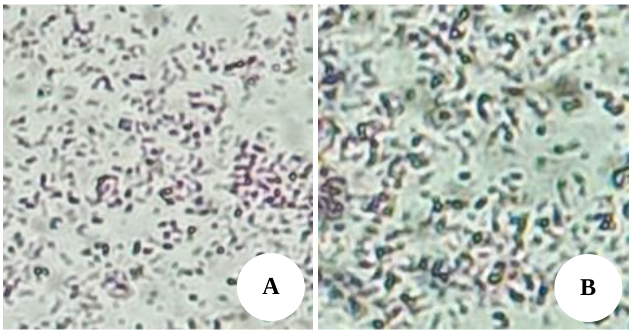

Figure 2. Gram staining of bacterial isolates: A. ISL1, B. ISL2 (400× magnification)

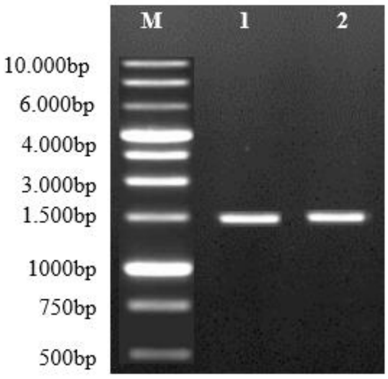

Figure 3. Electrogram profile of 16S rRNA gene on an agarose gel. Note: M: 1kb DNA ladder marker; 1. ISL1, 2. ISL2

The BLAST results on ISL1 and ISL2 isolates by matching with the results of the sequence on the Bank gene, each obtained 5 types of bacteria, all of which came from the *Vibrio* genus, which had similarities with the isolated isolates (Tabel 2). The results are based on the score showing a value of 100%, the quary cover showing the number 100%, the percentage identity in the range of 99.58% - 100%, and the e-value showing a value of 0.0. According to Dasilva et al. (2013), the level of sequence similarity for the genus level is the percentage identity value of 93% - 97%, while more than 97% represents the similarity at the species level.

On the ISL2, the 16S rRNA gene sequence was reconstructed in a phylogenetic tree to determine the relationship between ISL 1 and ISL2 isolates with those available in the NCBI gene bank. The ISL1 isolate is very closely related to *Vibrio parahaemolyticus* strain 11-1 (relationship level 99%), and ISL2 is closely related to *V. parahaemolyticus* strain B2-1 (relationship level 86%) (Figure 4).

Discussion

The results of the *Vibrio* calculation in this present study showed that the *Vibrio* population in both ponds tended to increase during the observation, with the highest total *Vibrio* reaching 2.7×10^4 CFU/mL. These results have exceeded the maximum limit set based on PERMEN KP Number: 75/PERMEN-KP/2016 concerning general guidelines for shrimp enlargement, namely 1.3×10^3 CFU/mL. In the isolation of *Vibrio* using TCBSA media, two different colonies were shown from the green and yellow color, each with different characteristics. Mailoa and Selta (2011) revealed that *Vibrio* colonies were yellow on TCBS Agar selective media because these bacteria were able to ferment sucrose. Conversely, bacteria that were unable to ferment sucrose grew into green colonies. In the gram stain, *Vibrio* bacteria are included in gram-negative bacteria because the colonies are red and have the shape of a bent rod (comma).

Molecular identification showed that ISL1 and ISL2 isolates were *Vibrio* bacteria indeed, and the ISL1 and ISl2 isolates were closely related, with a relationship rate of 72%. These two isolates were closely related because they had the same ancestor but underwent different changes when they evolved. The ISL1 and ISL2 isolates have a living environment that tends to be the same in temperature, salinity, pH, dissolved oxygen, etc. The ISL1 isolate was closely related to *V. parahaemolyticus* strain 11.1 (Acc. MZ081633.1) with a 99% relationship rate and 100% similarity index. In a previous study by Husna (2021), the bacterium *V. parahaemolyticus* strain 11.1 was isolated from ballast water in Tanjung Emas Harbor, Semarang.

Meanwhile, the ISL2 isolate was closely related to *V. parahaemolyticus* strain B2-1 (Acc. MK102629.1), with a relationship rate of 86% and a similarity index of 99.58%. The *V. parahaemolyticus* strain B2-1 was previously isolated from tuna samples (Rachmawati and Triwibowo 2018), which is supported by the opinion of Seprianto et al. (2017). Namely, the presence of the same species can be found in different environmental conditions because bacteria are organisms that can adapt to different physiological conditions quickly, have a fairly long dormant period, and have the possibility of forming endospores that can withstand extreme environmental conditions.

Rosnah (2015) reported that *V. parahaemolyticus* bacteria are rarely found in water below 10°C, and their presence increases with increasing water temperature. Zulkifli et al. (2009) and Kusmawarti et al. (2016) reported that high water temperatures are a factor in the high percentage of *V. parahaemolyticus* and the incidence of pathogenic *V. parahaemolyticus* tends to increase in the dry season when the pond water temperature is 31.69°C and pH

8.44. Soto-Rodriguez et al. (2015) reported that in 2013, atypical shrimp deaths in ponds in northwest Mexico occurred mainly in the first days after stocking, identified as a result of infection with *V. parahaemolyticus*. Characteristics of infection caused by *V. parahaemolyticus* are the occurrence of anorexia, lethargy and discoloration of the hepatopancreas, and damage to the hepatopancreas, which is characterized by severe necrosis of the tubular epithelium.

Different conditions can also affect the diversity of *Vibrio* in an area where there is competition for food and *Vibrio* interactions with other species found in a pond (Yital et al. 2007). Water quality conditions are closely related to physicochemical conditions and become a limiting factor for the growth of *Vibrio* bacteria in the pond ecosystem. Water physicochemical factors and nutrient availability influence the population of *Vibrio* sp. closely related to its growth ability.

Table 2. The results of the blast sequence of the 16S rRNA gene isolate ISL1 and ISL2

Description	Result links						
	Max score	**Total score**	**Query cover**	**E value**	**Per. ident**	**Accession**	**Reference**
Vibrio alginolyticus strain 2.2 16s ribosomal gene, partial sequence	2621	2621	100%	0.0	100%	MZ081632.1	Iksiroh et al. (2021)
Vibrio alginolyticus strain M.13-18928-16B 16S ribosomal RNA gene, partial sequence	2621	2621	100%	0.0	100%	KY229846.1	Go et al. (2016)
Vibrio alginolyticus strain M.14-00267-3B 16S ribosomal RNA gene, partial sequence	2619	2619	100%	0.0	100%	KY229787.1	Go et al. (2016)
Vibrio parahaemolyticus strain 11.1 16S ribosomal RNA gene, partial sequence	2615	2615	100%	0.0	100%	MZ081633.1	Iksiroh et al. (2021)
Vibrio parahaemolyticus strain SC33 16S ribosomal RNA gene, partial sequence	2621	2621	100%	0.0	100%	MK308610.1	Santoro et al. (2018)
Vibrio parahaemolyticus strain BF-1 16S ribosomal RNA, partial sequence	2621	2621	100%	0.0	99.65%	KR137715.1	Subraamaniam and Kasturi (2015)
Vibrio parahaemolyticus strain V2 16S ribosomal RNA gene, partial sequence	2621	2621	100%	0.0	99.65%	OL850009.1	Chahouri et al. (2021)
Vibrio diabolicus strain SC11 16S ribosomal RNA, partial sequence	2595	2595	100%	0.0	99.58%	MK308588.1	Santoro et al. (2018)
Vibrio alginolyticus strain V10A 16S ribosomal RNA gene, partial sequence	2597	2597	100%	0.0	99.58%	MG262456.1	Serano et al. (2017)
Vibrio parahaemolyticus strain B2-1 16S ribosomal gene, partial sequence	2597	2597	100%	0.0	99.58%	MK102629.1	Fang et al. (2018)

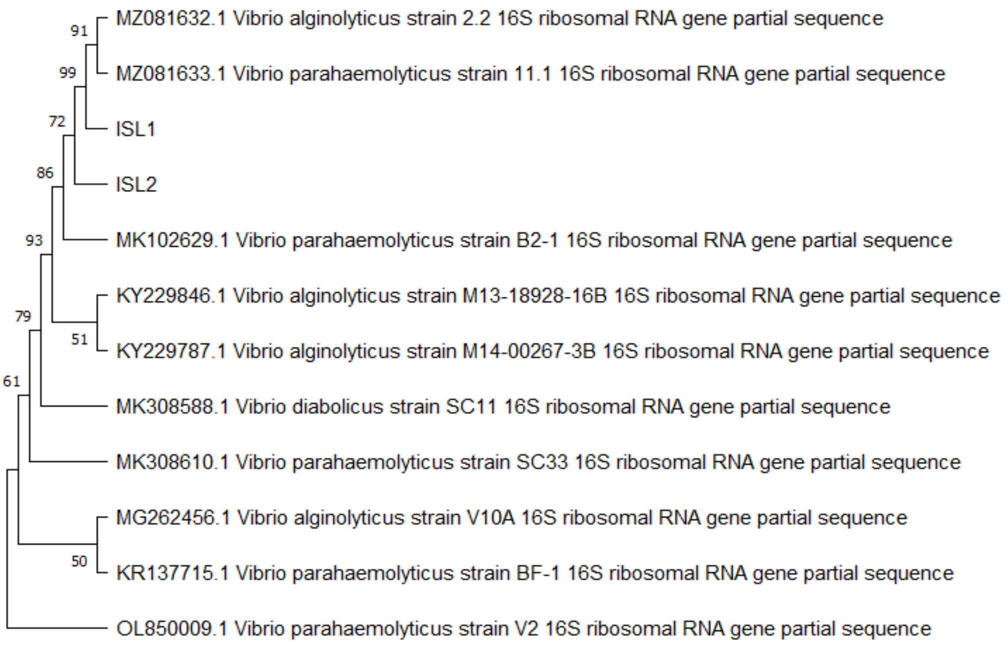

Figure 4. Phylogenetic tree of ISL and ISL2 sequences using the Neighbor-Joining algorithm as a result of sequencing with a 1000 × bootstrap

The increase in ammonia concentration in the rearing media was due to the accumulation of organic materials such as leftover feed with high protein content and feces, as well as waste from shrimp metabolism, which was secreted through the gills. According to Chatterjee et al. (2014), *Vibrio* utilizes ammonia as a nitrogen source for the source of energy and other organic compounds for the element in the formation of protoplasmic cells, especially in the formation of cell walls. Jawetz et al. (2016) also stated that bacteria need nitrogen to synthesize proteins, DNA and RNA.

The nitrate concentration in both ponds was low at 0-1 mg/L but exceeded the normal limit for total nitrate in shrimp rearing media. The presence of nitrate in shrimp pond waters is due to nitrate levels in ponds derived from nitrite oxidation by Nitrobacter bacteria. Therefore, the amount of nitrite and ammonia can determine nitrate levels in shrimp ponds. In addition, nitrate is an important N component utilized as a nutrient acceptor by *Vibrio* (Wang et al. 2019).

Salinity levels also influence the increase in the *Vibrio* population in ponds. Salinity measurement in ponds obtained is high in the range of 20-27 ppt, while *Vibrio* is halophilic or can live in environmental conditions with high salt content. According to Pariakan and Rahim (2021), salinity strongly influences the presence of *Vibrio* sp., which is equal to 0.0302, and the total bacteria was found to be higher at higher salinity conditions. Environments with high salt content have low water activity and high concentrations of inorganic ions. Halophilic bacteria accumulate compatible solutes to protect metabolic activity and prevent water loss from cells in high salinity (2%-30%) habitats (Arisandi et al. 2017).

Temperature affects the metabolic activity of organisms which causes the spread of organisms in the waters to be limited by the temperature of the waters. The pond measurement water temperature is obtained in the range of 30-33°C. Lailatussyifa et al. (2020) reported that *Vibrio* bacteria can grow at an optimum temperature of 25°C but can still survive and grow well at 37°C. Arivo and Annisatusholeha (2017) reported that temperature plays a role in bacterial growth, synthesis speed, and enzyme activation. An increase in temperature causes an increase in the kinetic energy of the reactants. Temperatures that are too low can inhibit the activity of enzymes, while temperatures that are too high can cause enzyme denaturation.

pH is an important factor in the growth of *Vibrio*. The pH of the pond water was measured in the range of 7.9-8.2 and was still within the tolerance range for *Vibrio* growth. The optimum pH for *Vibrio* growth is 7-7.5, which can still grow up to pH 9 (Ligthner et al. 1992; Hikmawati et al. 2019). The effect of pH on bacterial growth depends on the enzymes needed to catalyze metabolic reactions associated with bacterial growth. Low or high pH can cause denaturation, which can inhibit enzyme activity and have an impact on decreasing the number of bacterial growth (Arivo and Annisatusholeha 2017).

Furthermore, dissolved oxygen in water can also increase bacteria in pond waters because *Vibrio* is a facultative anaerobic bacterium. The measurement results showed that the dissolved oxygen content in both ponds was high on the third day, namely 32.4 mg/L in pond 1 and 38 mg/L in pond 2, and dissolved oxygen decreased until the 9th day of observation, namely 15.5 mg/L in pond 1 and 26.7 mg/L in pond 2. Yuhantaka (2018) reported that *Vibrio* bacteria are facultative anaerobic bacteria which means they can live and grow using or without oxygen. According to Ariadi et al. (2021), it is described that dissolved oxygen has a negative relationship with the total abundance of bacteria, which means that when there is an increase in the total number of bacteria, there is a decrease in the concentration of dissolved oxygen.

Under aerobic conditions, *Vibrio* utilizes oxygen as an electron acceptor and uses oxygen in aerobic respiration. In aerobic conditions, *Vibrio* can metabolize perfectly; one mole of glucose will be converted into 36 ATP. Compared to anaerobic conditions, *Vibrio* will carry out anaerobic metabolism; one glucose molecule is converted into 2 ATP (Suryani, 2022). That causes *Vibrio* in aerobic conditions to have greater energy in the growth and reproduction process, so it has an impact on increasing the population of *Vibrio* sp. in ponds. Moreover, the diversity of *Vibrio* bacteria in waters is caused by several biotic and abiotic factors to maintain their life (Ilmiah et al. 2012). Therefore, it is necessary to increase the intensity of water changes and efforts to control water quality to maintain dissolved oxygen levels and manage organic matter content in ponds to prevent the increase of pathogenic bacteria, especially *Vibrio*.

ACKNOWLEDGEMENTS

Thanks to all those who have helped, especially to the Assistant for Biology Laboratory, Universitas Negeri Gorontalo, Indonesia, who has provided facilities, tools, and materials to implement this research.

REFERENCES

Ariadi H, Wafi A, Musa M, Supriatna S. 2021. Correlation between water quality parameters in intensive cultivation of white shrimp (*Litopenaeus vannamei*). Samakia: J Fish Sci 12 (1): 18-28. DOI: 10.35316/jsapi.v12i1.781.

Arisandi A, Wardani MW, Kaswan B, Garina DA. 2017. Impact of salinity differences on viability of *Vibrio fluvialis* bacteria. Sci J Fish Mar Affairs 9 (2): 91-97. DOI: 10.20473/jipk.v9i2.7636.

Arivo D, Annisatuussholeha N. 2017. Effect of osmotic pressure pH and temperature on the growth of *Escherichia coli* bacteria. J Med Health Sci 4 (3): 152-163.

Chahouri A, Radouane N, Yacoubi, Moukrim A, Banaoui A. 2021. Occurrence and Distribution of Fecal Indicator Bacteria, *Salmonella* and *Vibrio* Species in the Agadir Bay Ecosistems (Soutern Moroco) Influence of Environmental parameters. Plant Protections, Ecole National d'agriculture, Moroco.

Chatterjee SN, Syed AA, Mukhopandhyay B. 2014. Diversity of soil bacteria in some village areas enjoyning to Joypur Forest of Vankura District of West Bengal, India. Intl J Environ Biol 4 (1): 67-70.

Dasilva MAC, Cavalett A, Spinner A, Rosa DC, Jasper RB, Quecine MC, Bonatelli ML, Pizzirani-Kleiner A, Corcao G, De Souza Lima AO. 2013. Phylogenetic identification of marine bacteria isolated from deep-sea sediments of the Eastern South Atlantic Ocean. Spinger Plus 2: 127. DOI: 10.1186/2193-1801-2-127.

Fang H, Wang X, Guo Z. 2016. Isolation, Identification and Bioloycal Characteristics of *Vibrio*. Animal Science and Tecnology, Shadong Agricultural University, Tiongkok.

Fatmala I, Pranggono H, Linayati. 2019. Identifikasi bakteri *Vibrio* sp. dalam hepatopankreas udang Vannamei (*Litopenaeus vannamei*) pada tambak yang diberi probiotik di Tambak Sampang Tigo Kelurahan Degau Kota Pekalongan. J Litbang Kota Pekalongan 16: 42-48. DOI: 10.54911/litbang.v16i0.95. [Indonesian]

Ganesh EA, Das S, Chandrasekar K, Arun G, Balamurugan S. 2010. Monitoring of total heterotrophic bacteria and *Vibrio* spp. in an aquaculture pond. Curr Res J Biol Sci 2 (1): 48-52.

Go J, Deutscher AT, Spiers Z, Dahle K, Kirkland PD, Jenkins C. 2016. An Investigations Into Mass Mortalities of Unknown aetiology in Pasific Oysters, Crassosstrea Gigas, in Port Stephens, New South Wales, Australia. Elizabeth Macarthure Agricultural Institute Departement of Primary Industries, Australia. DOI: 10.3354/dao03146.

Haliman RW, Adijaya DD. 2005. Udang Vanamei, Pembudidayaan dan Prospek Pasar Udang Putih yang Tahan Penyakit. Penebar Swadaya, Jakarta. [Indonesian]

Hikmawati F, Susilowati A, Ratna S. 2019. Amount detection and pathogenicity test of *Vibrio* spp. in green shells (*Perna viridis*) in the Yogyakarta Beach Tourism area. J Pros Sem Nas Masy Biodiv Indones 5 (2): 334-339. DOI: 10.13057/psnmbi/m050234.

Husna IE. 2021. Isolation Ballast Water Bacteria from Tanjung Mas Port. Marine Science, Universitas Diponegoro, Semarang. [Indonesian]

Idami Z, Nasution RA. 2020. The abundance of *Vibrio* sp. based on the location of shrimp farming in Pidie Regency. Biomes: J Biol Learn Biol 5 (2): 121-134. DOI: 10.32528/bioma.v5i2.4012.

Iksiroh HE, Anggoro S, Abdurachim HR, Subagiyo S, Ighwerb MI. 2022. Bacteriological study of ballast water at Tanjung Emas Port, Semarang. Ilmu Kelautan: Indones J Mar Sci 27 (1): 45-52. DOI: 10.14710/ik.ijms.27.1.45-52.

Ilmiah S, Widanarni EH. 2012. Isolation and characterization of Pathogenic *Vibrio* on Tiger grouper *Epinephelus fuscoguttatus*. Indones Aquac J 11 (1): 28-37. DOI: 10.19027/jai.11.28-37.

Indonesian National Standard (SNI). 2015. SNI 2332.3:2015. Determination of Total Plate Number (ALT) in Fishery Products. Directorate General of Fisheries. Jakarta.

Jawetz E, Melnik J, Adelberg E. 2016. Medical Microbiology Edition 25: EGC, Jakarta.

Jayasree L, Janakiram P, Madhavi R. 2006. Characterization of *Vibrio* spp. associated with diseased shrimp from culture ponds of Andhra Pradesh (India). J World Aquac Soc 37 (4): 523-532. DOI: 10.1111/j.1749-7345.2006.00066.x.

Kusmawarti A, Hermana I, Yennie Y, Wibowo S. 2016. The presence of pathogetic *Vibrio parahaemolyticus* in pond shrimp originating from the North Coast of Java. J Mar Fish 11 (1): 41-54. DOI: 10.15578/jpbkp.v11i1.285.

Lailatussyifa A, Widyorini N, Jati OE. 2020. Analysis of total bacteria *Vibrio* sp. in sediments at different mangrove densities at Ujung Piring Beach, Jepara. Sea Sand J 4 (1): 1-8.

Ligthner DV, Bell TA, Redman RM, Mohney LL, Natividad JM, Rukyani A, Poernormo A. 1992. A review of some major diseases of economic significance in penacids prawn/Shrimps of the America and Indo pacific, In. Shariff M, Jr Arthur, Subasinghe RP (eds). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila.

Mailoa MC, Setha B. 2011. Pathogenic characteristics of *Vibrio* sp. isolated from eel mucus (*Anguilla* Sp.). Molucca Medica 4 (1): 42-48.

Mika AM, Golebiowski M, Skorkowski, F.E., & Stepnowski, P. 2013. Composition of fatty acids and sterols composition in brown shrimp *Crangon crangon* and herring *Clupea harengus* membrans from the Baltic sea. Oceanol Hydrobiol Stud 41 (2): 57-64. DOI: 10.2478/s13545-012-0017-z.

Pantjara B, Nur H. 2014. Study of Pond Bacteria Population in Integrated Multitrophic Aquaculture (IMTA) Shrimp Cultivation System. XI Annual National Seminar on Fisheries and Marine Research Results. Universitas Gadjah Mada, Yogyakarta. [Indonesian]

Pariakan A, Rahim. 2021. Characteristics of water quality and presence of *Vibrio* sp. in the traditional shrimp pond area on the Wundulako and Pomalaa Kolaka Coastal Areas. J Fish Mar Res 5 (3): 547-556. DOI: 10.21776/ub.jfmr.2021.005.03.5.

PERMEN-KP: Number: 75/PERMEN-KP/2016 concerning General Guidelines for Growing Windu Shrimp (*Panaeus monodon*) and Vaname Shrimp (*Litopanaeus vannamei*).

Rachmawati N, Triwibowo R. 2021. Skrining dan identifikasi bakteri pembentuk histamin yang diisolasi dari tuna, tongkol, dan cakalang segar di Wilayah Jawa Barat, DKI Jakarta, dan Banten. J Pascapanen dan Bioteknologi Kelautan dan Perikanan 16 (2): 93. DOI: 10.15578/jpbkp.v16i2.771. [Indonesian]

Rosnah. 2015. Analysis of *Vibrio parahaemolyticus* on Vaname Shrimp (*Litopanaeus vannamei*) Based on Maintenance Location and Harvest Age. [Skripsi]. Pangkajene State Agricultural Polytechnic and Pengkep Archipelago. [Indonesian]

Santoro EP, Messias CS, Vilela CL, Vilela HD, Peixoto RS. 2018. Microbial Community of Super Corals from Bahia Brazil. General Microbiology, Federal University of Rio de Janeiro.

Sarjito, Apriliani M, Afriani D, Haditomo AHC. 2015. Agent causing *Vibrio*sis in intensively cultured vaname (*Litopanaeus gariepinus*) shrimp in Kendal. J Kelautan Tropis 18 (3): 189-196. DOI: 10.14710/jkt.v18i3.533. [Indonesian]

Satomayor MA, Reyes JK, Restrepo R, Borbor CJ, Maldonado M, Bayot B. 2019. Efficacy assessment of commercially available natural products and antibiotics, commonly used for mitigation of pathogenic *Vibrio* outbreaks in Ecuadorian Penaeus (*Litopenaeus vannamei*) hatcheries. Plos ONE 14 (1): 1-19. DOI: 10.1371/journal.pone.0210478.

Serrano W, Tarazona UI, Olaechea RM, Gonzales J, Valle S. 2017. Culturable Gut Microbiota Diversity in the Scallop Argopcten Purpuratus From two Shellfish Growing areas in Peru. Facultad de Ciancias Veterinarias Biologycas. University Cientifica Del Sur.

Soto-Rodriguez SA, Gill BG., Rodolfo LO, Betancourt-Lozano M, Morales-Covarrubias MS. 2015. Field and experimental evidence of *Vibrio parahaemolyticus* as the causative agent of acute hitopancreatic necrosis disease of cultured shrimp (*Litopanaesus vannamei*) in Nothwesern Mexico. Appl Environ Microbiol 81 (5): 1689-1699. DOI: 10.1128/AEM.03610-14.

Subramaniam N, Kasturi. 2015. Biofouling Bacteria from Boast. Microbiology Etiraj Collage for Women. Chenai, India.

Supono. 2018. Manajemen Kualitas Air untuk Budidaya Udang. Anugrah Utama Raharja, Bandar Lampung. [Indonesian]

Suryani Y. 2022. Physiology of Microorganisms. Gunung Djati Publishing, Bandung. [Indonesian]

Wahyuni Y, Jamilah IT, Suryanto D. 2017. Isolation of opportunistic pathogenic bacteria from shrimp ponds of Sumatra Utara. Agrohita J 1 (2): 71-75. [Indonesian]

Wang H, Zeuchner J, Eremets M, Troyan I, Wiliam J. 2019. Stable Solid and aqueonus H_2CO_3 from CO_2 and H_2O at high pressure and high temperature. Sci Rep 6: 1-8: DOI: 10.1038/srep19902.

Yital M, Peter HF, Hammes F, Egli T. 2007. Growth of *Vibrio cholerae* Ol Ogawa Eltor in freshwater. Microbiology 153: 1993-2001. DOI: 10.1099/mic.0.2006/005173-0.

Yuhantaka N. 2018. Identification of *Vibrio cholera* bacteria in shrimp paste without addition and with the addition of red dragon fruit peel extract (*Hylocereus polyrhizus*) as a natural dye. Stikes Medika Medika Insan Jombang 10 (1): 279-288. [Indonesian]

Zhou J, Fang W, Yang X, Zhou S, Hu L, Li X, Qi X. 2012. A nonluminescent and highly virulent *Vibrio harveyi* strain is associated with bacterial white tail disease of *Litopenaeus vannamei* shrimp. 7 (2): 19-22. DOI: 10.1371/journal.pone.0029961.

Zulkifli Y, Alitheen NB, Son R, Yeap SK, Lesley MB, Raha AR. 2009. Identification of *Vibrio parahaemolyticus* isolates by PCR targeted to the toxR gene and detection of virulances genes. Intl Food Res J 5 (16): 289-296.