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The Effectiveness of β-Glucan *Saccharomyces cerevisiae* and Endophytic Bacteria *Sonneratia alba* Leaves on the Immune Response of *Litopenaeus vannamei* Infected with *Vibrio harveyi*

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ABSTRACT

Vibrio harveyi can cause mass death in vaname shrimp. The β-Glucan content in *Saccharomyces cerevisiae* can stimulate the immune system non- specifically. Meanwhile, the endophytic bacteria *Sonneratia alba* has antibacterial activity. This study aims to determine the effectiveness of the probiotic fee formulation of β-Glucan *S. cerevisiae* using a combination of antibacterial S. *alba* leaves as an effort to inhibit the growth of *V. harveyi* in vaname shrimp (*L. vannamei*). The research method used is an experimental probiotic formulation from a combination of β-Glucan *S. cerevisiae* and endophytic bacteria *S. alba* through shrimp feed with 6 treatments and 4 replications, The parameters used are Total Haemocyte Count, Differential Haemocyte Count and Survival Rate. This study was conducted in vivo tests for 10 days of probiotic feed maintenance and 7 days of infection with a total research period of four months at the Faculty of Health, Medicine, and Natural Sciences, Airlangga. The results of the research show that the combination of ingredients can inhibit the growth of *V. harveyi* through the immune response and survival of vannamei shrimp. The best treatment was P3 with a Total Haemocyte Count (THC) of 7,54x10^6 cells/ml and Differential Haemocyte Cout (DHC) (60,56% granular, 16,68% semi-granular, 22,76% hyaline) with 85% survival. The provision of probiotics had a real effect and became the optimum formulation to inhibit the growth of *V. harveyi*.

Keyword: β-Glucan *S. cerevisiae*, endophytic bacteria *S. alba*, *V. harveyi.*

INTRODUCTION

Aquaculture in Indonesia has many promising fishery commodities, one of which is vaname shrimp (*Litopenaeus vannamei*). In 2024, the Ministry of Maritime Affairs and Fisheries (KKP) targets national shrimp production of 2 million tons per year. Shrimp production has increased by 21.25% in 2021-2022. Shrimp production in 2021 reached 1.22 million tons worth IDR 77.02 trillion, while in 2022 it was 1.48 million tons worth IDR 92.69 trillion (KKP, 2022). This increase is also projected to continue to increase as the demand for shrimp is getting higher.

Vibrio harveyi bacteria is one of the causes of vibriosis disease in shrimp from the larval stage to adulthood. Vibriosis is very dangerous because it can cause mass death of shrimp up to 100% and attacks when the shrimp are weak and environmental factors are extreme (Lopillo, 2000) This results in economic losses and reduces the quantity and quality of *L. vannamei* production. Clinical symptoms of shrimp vibriosis are: black spots or lines on the lateral cephalothorax, opaque white muscles, and smoky body coloration (Evan *et al.*, 2021). *V. harveyi* attacks on *L. vannamei* are very detrimental to farmers, so it is necessary to suppress *V. harveyi*. Bacterial resistance can

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occur due to mutation and random load selection and antibiotics act as selection agents, so that it is possible for the multiplication of resistant bacterial groups and suppressing the growth of bacteria that are sensitive to antibiotics. Administration of antibiotics and immunostimulants is less effective because it causes resistant bacteria and leaves residues (Takwin *et al.,* 2022).

According to Fitri *et al.* (2018) *Avicennia alba* mangrove leaves are able to inhibit the growth of *V. harveyi* in *L. vannamei*. However, the inhibition strength is still low. Sponge *Aaptos aaptos* can also inhibit the growth of *V. harveyi*, but the survival rate value is only 50% (Rosmiati *et al.,* 2020). Jatropha leaf (*Jatropha curcas*) extract can increase the immune system of *L. vannamei* infected with *V. harveyi*, but the survival rate is still higher without Jatropha leaf extract (Takwin *et al.,* 2022). For this reason, alternative *V. harveyi* antibacterial materials are needed that are more effective.

Sonneratia alba is one of six species of mangrove plants that grow on the coast of Banyuwangi. *S. alba* has secondary metabolites in the form of: phenolics, triterpenoids, flavonoids, saponins, tannins, and alkaloids (Aulia & Sulistyaningsih, 2019). Research by Syafitri *et al.* (2021) showed that the secondary metabolites of *S. alba* ethanol extract have the highest inhibitory power on gram-positive and negative bacteria compared to other mangrove types. Endophytic bacteria offer advantages including being able to be produced on a large scale, allowing new bioactive components to be obtained by providing different conditions and are commonly found in mangrove plants. Endophytic bacteria are microbes that are in symbiotic mutualism with their host plants.

Suryani & A'yun (2022) isolated endophytic bacteria from *S. alba*, but no antibacterial application has been carried out on *V. harveyi*. Thus, to optimize the potential of active ingredients *S. alba* as an antibacterial *V. harveyi*, isolation and application of endophytic bacteria from *S. alba* leaves will be carried out. *S. cerevisiae* is a yeast that has a high nutritional content such as protein, carbohydrates, fats, and minerals so that it is widely used as a feed ingredient (Panab *et al.,* 2020). The cell wall of *S. cerevisae* can synthesize the essential substance β-Glucan which can stimulate non-specific immunity (Lei *et al.,* 2021). Ayiku *et al.* (2020) reported the potential of probiotic feed from *S. cerevisiae* as an immunostimulant for shrimp infected with *V. harveyi*.

The use of *S. cerevisiae* does not leave residues in the body or the environment and is not harmful to health if consumed. The mechanism of Immunostimulant β-Glucan *S. cerevisiae* by activating white blood cells (leukocytes) which function to stimulate hemocytes to degranulate and proteins that will be released such as molecular bonds so that organisms are more resistant to viral, bacterial, fungal and parasitic infections (Darwanti dkk., 2016). The method of administering probiotics mixed with feed will improve feed quality, hydrolyze feed and undergo a fermentation process by microbes before being eaten. Therefore, the innovation of *S. cerevisae* as a feed source as well as an immunostimulant has the potential to be combined with endophytic bacteria *S. alba* as an effective formulation in suppressing the growth of *V. harveyi*. Based on the above background, the application of probiotics made from immunostimulant β-Glucan *S. cerevisiae* combined with antibacterial *S. alba* leaves aims to inhibit *V. harveyi* in vaname shrimp through improving the immune system and survival.

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MATERIALS AND METHODS

Study Period and Location

This research was conducted over three months (April-July). Isolation and testing at the Infection Laboratory and Feed Laboratory of FIKKIA, Airlangga University. *S. alba* leaves were collected from Santen Island Beach, Karangrejo, Banyuwangi, East Java. *L. vannamei* were collected from intensive ponds in Pakis Village, Banyuwangi, East Java.

Ethical Approval

This study followed the principles of use and principles of animal welfare and has received approval from the Research Ethics Commission of the Center for Life Sciences Laboratory, Brawijaya University Number 191/LSIH/EP/2024.

Tools and Materials

The materials used in this research are *S. alba*, *S. cerevisiae* mold, distilled water, *L. vannamei*, *V. harveyi* isolate, Nutrient Broth (NB), Nutrient Agar (NA), Tryptone Soya Agar (TSA), Sabouraud Dextrose Agar (SDA), Thiosulphate Citrate Bilesalt Sucrose (TCBS), Phosphate Buffered Saline (PBS), physiological NaCl, Plate Count Agar (PCA), Mueller Hinton Agar (MHA), Triple Sugar Iron Agar (TSIA), Sulfide Indole Motility (SIM), oxidase strip, hydrogen peroxide, oxidative fermentative, gram staining material, syringe, dropper pipette, microtip, CH_3COOH, NaOH, paper disk, label paper, $KMnO_4$, methanol, giemsa, ammonia, nitrite and nitrate kit. The tools used in this research are petri dishes, test tubes, bunsen, ose needles, round ose, micropipettes (Dragon lab, China), erlenmeyer (100 ml and 250 ml) onemed (Indonesia), laminary air flow, measuring cup, hot plate (C-MAG HS7, German), autoclave (Hirayama, Japan), incubator (Memmert IN 55, German), centrifuge, mortar and pestle, binocular microscope (Olympus CX23, Japan), haemocytometer (German), caliper, test tube rack, volume pipette, refractometer, thermometer, pH meter, analytical balance (ohaus PX223, America), ruler, object glass, cover glass, aquarium, aerator, aerator hose and airstone.

Isolation of Endophytic Bacteria of S. alba Mangrove Leaves

The procedure was adopted from Yanti *et al.* (2021) which has been modified. *S. alba* leaves that were old enough were selected using purposive sampling method from 4 location points with certain considerations. Leaf samples were cleaned with alcohol and dried. Next, mashed with a sterile mortar and pestle. Weighed as much as 1 g, put in a test tube containing 0.9% NaCl as much as 10 ml and vortexed (serial dilutions up to 10^{-5}). Then each dilution was inoculated into TSA media with the spread plate method as much as 100 µL and incubated at 37ºC, 24 hours. The growing bacterial colonies were purified by the quadrant method on TSA media. Bacterial morphology was observed macroscopically and microscopically.

Biochemical Test of Endophytic Bacteria

Biochemical tests used to identify the genus of bacteria include catalase test, oxidase test, Triple Sugar Iron Agar (TSIA) test, Sulfide Indole Motility (SIM) test and oxidative fermentative (OF) test.

Colony Counting of Endophytic Bacteria of Sonneratia alba Mangrove Leaves

Take 1 ose of bacteria was inoculated into 0.9% NaCl solution, homogenized (serial dilutions up to 10^{-5}). Each dilution was taken 100 µL and inoculated on PCA media with the spread plate technique. Then incubated at 37°C, 24 hours. The number of bacterial colonies was counted in Colony Forming Units Per ml (CFU/ml) (Suryani and A'yun, 2022).

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Extraction of β-Glucan S. cerevisiae

A 30 mL culture sample was centrifuged at 7000 rpm for 20 minutes at 15°C. The supernatant was discarded, the pellet was added 5 mL of 3% NaOH, heated for 5 hours at 90°C. Then centrifuged at 5000 rpm for 10 minutes. CH_3COOH was added until the pH of the solution was 6.8-7. Precipitated with 3 mL ethanol, then centrifuged at 5000 rpm for 10 minutes. The separated pellet was dried then (Cempaka, 2015).

V. harveyi Culture and Total Plate Count

V. harveyi bacteria used were obtained from the Laboratory of the Center for Brackish Water Aquaculture (BBPBAP) Jepara. *V. harveyi* culture was taken 1 round ose, transferred into NB media and incubated for 24 hours at 37°C. Furthermore, it was isolated on TCBS media with the spread plate method, incubated for 24 hours at 37°C and then counted the colonies (Seniati *et al.,* 2019).

Antibacterial Activity Test against V. harveyi

V. harveyi bacteria were rejuvenated on NA media the day before the test, then inoculated in NB until the density was equivalent to 0.5 McFarland. Pathogenic bacteria were then inoculated into MHA media by the method of spreading paper disks with a size of 6 mm that had been soaked in β-Glucan *S. cerevisiae* and endophytic bacteria of *S. alba* leaves and then placed on MHA media. placed on MHA media and incubated for 24 hours at 37°C (Seniati *et al.,* 2019).

Feed Mixing

β-Glucan *S. cerevisiae* and endofit bacteria *S. alba* were mixed according to the dose variation, homogenized for 3-5 minutes, sprayed on shrimp pellets. Next, shrimp pellets were mixed with 2% egg white (Pardede *et al.,* 2024). Dried for 10-15 minutes. Feed nutrition: 35% protein, 5% fat, 12% water, 15% ash, and 4% crude fiber.

In Vivo Testing

In vivo testing with 30 day old *L. vannamei* test animals. *L. vannamei* samples weighing around 2-2.4 g/tail and 6.1-6.3 cm/tail in length as many as 180 were divided into three groups, namely the positive control group, negative control and treatment group with four replications, 10 days of probiotic feed maintenance and 7 days of infection period. P1 (negative control) = not feed SC + endophytic bacteria and injected with 0,1 ml of PBS, P2 (positive control) = not feed SC + endophytic bacteria and injected with *V. harveyi* at a concentration of 10^6 CFU/ml at 0.1 ml, P3 = SC: endophytic bacteria (1:1), P4 = SC: endophytic bacteria (1:2), P5 = SC: endophytic bacteria (2:1), and P6 = SC: endophytic bacteria (2:2). SC: endophytic bacteria (2:2) were each injected with *V. harveyi* at a concentration of 10^6 CFU/ml (LC50) at 0,1 ml. Maintained and observed for clinical symptoms after injection or challenge test.

Measurement parameters

Total Hemocyte Count (THC)

Hemolymph was collected (0.1 ml) from the base of the walking leg in all treatments using a 1 ml syringe with 0.2 ml of anticoagulant, then homogenized for 5 minutes before being dripped into a hemocytometer for cell counting. THC observation refers to the method of Balxhalll and Dalishley (1973) with the formula:

$$Total\ hemocytes = \sum hemocytes\ counted \times diluting\ factor \times 10^4$$

Differential Hemocyte Count (DHC)

Hemolymph was placed on an object glass, allowed to air dry, fixed with methanol for 5-10 minutes, stained with Giemsa solution for 15-20 minutes, then washed and dried before calculating the differential and total

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percentage of hemocytes. DHC observation refers to the method of Balxhalll and Dalishley (1973) with the formula:

$$\%\ hemocyte\ cell\ type = \frac{\sum hemocyte\ cell\ type}{total\ hemocytes} \times 100$$

Survival Rate, Growt Rate, and Water Quality

Survival rates were determined by comparing the number of *L. vannamei* at the start and end of the rearing period for each treatment with the formula (Effendie, 1997) as follows:

$$SR = \frac{Nt}{No} \times 100$$

Description:

N_t = the number of test shrimp that survived at the end of the observation

N_0 = the number of test shrimp that were spread at the beginning of the observation

Growth rates were assessed by measuring the weight and length of *L. vannamei* at both the beginning and end of rearing. Water quality was monitored daily for pH, temperature, and salinity, with ammonia, nitrite, and nitrate levels tested every other day.

Data analysis technique

The analytical method used in this research is descriptive analysis by summarizing the research results. Quantitative analysis of ANOVA and regression analysis to assess the functional effect between probiotic administration on the development of *V. harveyi* bacteria with different treatments. If the hemocyte data from THC and DHC had a significant effect, Duncan's further test was conducted to assess the best treatment.

RESULTS AND DISCUSSIONS

Isolation of *Sonneratia alba* Endophytic Bacteria

Based on the results (Table 1) of the biochemical test of *S. alba* endophytic bacteria, isolates of *Veillonella* sp. and *Acinetobacter* sp. were obtained. Based on their cell morphology, both isolates are gram-negative, indicated by pink samples because they contain more lipids and are round in shape, in line with Arikhah (2021). Isolates of *Veillonella* sp. and *Acinetobacter* sp. have similar characteristics in the form of: positive catalase test, TSIA test shown M/K (Red/Yellow) results, and OF test. However, the negative results of the oxidase test and motility which is indicated by the accumulation of bacterial colonies in the center (Damayanti *et al.,* 2018). Thus, it can be seen that the *S. alba* isolates, namely *Veillonella* sp. and *Acinetobacter* sp. are catalase positive, oxidase negative, fermentative, and motile which corresponds to research (Harpeni & Saefulloh, 2015; Kabatia *et al.,* 2023). The results of bacterial isolation on TSA media (Table 2) can then be calculated to determine the Total Plate Count (TPC) value which can be seen from Table 2. Based on the value of colony abundance in endophytic bacterial samples, for some dilutions it is declared unqualified because to calculate TPC, the number of colonies is >30-300 and if more than that it is categorized as too numerous to count (TNTC). Then the TPC results in sample 1A (*Veillonella* sp.) were $8.1x10^6$ CFU/ml and in sample 2A (*Acinetobacter* sp.) were 6.2×10^6 CFU/ml. The purpose of counting bacterial colonies is to determine the growth of bacteria and is used in determining research treatment.

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Table 1. Biochemical Test Results of Endophytic Bacteria *S. alba*

Sample	Gram Staining	Catalase	Oxidase	OF	SIM	TSIA	Genus
1A	negative, coccus	positive	negative	fermentative	non motile	basic	*Veillonella*
2A	negative, coccus	positive	negative	fermentative	non motile	acid	*Acinetobacter*
3A	negative, coccus	positive	negative	fermentative	non motile	basic	*Veillonella*
4A	negative, coccus	positive	negative	fermentative	non motile	basic	*Veillonella*

Table 2. Colony Count Results of *S. alba* Endophytic Bacteria

Genus	Dilution	Colony Count		TPC (CFU/ml)
		Test 1	Test 2	
Sample 1A *Veillonella* sp.	10^{-1}	TNTC	TNTC	$8{,}1\times10^{6}$
	10^{-2}	TNTC	TNTC	
	10^{-3}	TNTC	TNTC	
	10^{-4}	258	TNTC	
	10^{-5}	166	105	
Sample 2A *Acinetobacter* sp.	10^{-1}	TNTC	TNTC	$6{,}2\times10^{6}$
	10^{-2}	TNTC	TNTC	
	10^{-3}	TNTC	TNTC	
	10^{-4}	241	TNTC	
	10^{-5}	100	TNTC	

Isolation and Extraction of β-Glucan *Saccharomyces cerevisiae*

Isolation of β-Glucan *S. cerevisiae* was successfully carried out, indicated by the formation of a purplishblue color characteristic of gram-positive groups (Figure 1). Ida *et al.* (2022) reported that the blue-purple color is due to the structure of the cell wall of *S. cerevisiae* which is still very thin and getting thicker. In addition, it contains Glucan which has a strong affinity for crystal violet and iodine so that the gram staining results are purplish blue. *S. cerevisiae* itself contains the enzyme maltase which converts maltose into glucose (Puspita, 2020).

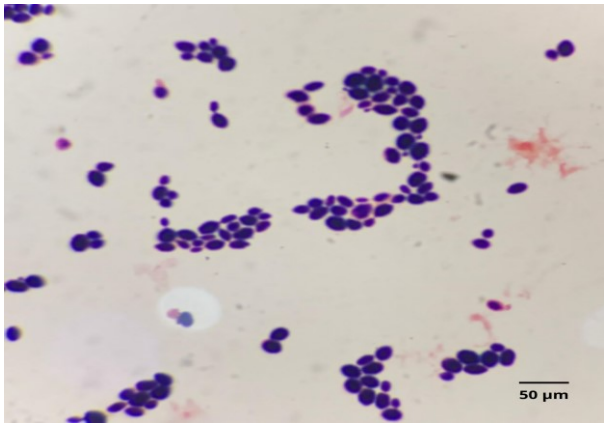

Figure 1. Isolation Result of β-Glucan *Saccharomyces cerevisiae*

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V. harveyi Bacterial Colonies

The results of pure culture of *V. harveyi* obtained a round colony shape, convex elevation and yellow color in Figure 2. It is in accordance with research Apriliani *et al.* (2016), that the *V. harveyi* bacteria have a yellow colony color, round colony shape, and convex elevation on TCBS media.

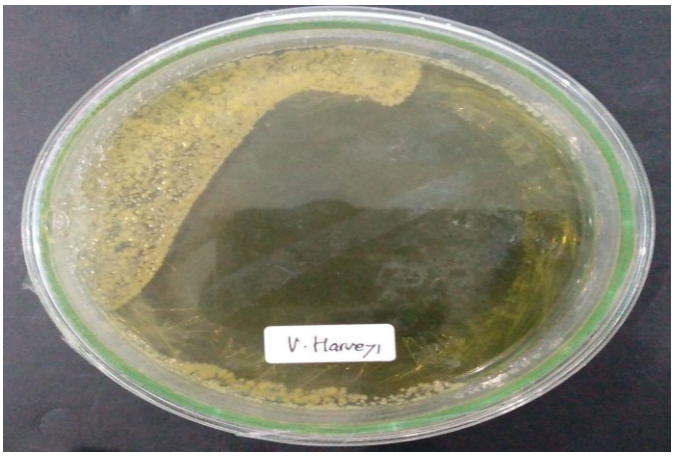

Figure 2. Isolation Result of *V. harveyi*

In Table 3 the number of *V. harveyi* bacterial colonies obtained was 10^7 CFU/ml (Table 3), to be used during the challenge test

Table 3. Calculation Results of *V. harveyi* Bacterial Colonies

Bacteria	Dilution	Colony Count		TPC (CFU/ml)
		Test 1	Cawan 2	
V. harveyi	10^{-1}	TNTC	TNTC	10^7
	10^{-2}	TNTC	TNTC	
	10^{-3}	TNTC	TNTC	
	10^{-4}	273	205	
	10^{-5}	186	174	

Antibacterial Activity Test against *V. harveyi*

The results of the antibacterial activity test showed that *Acinetobater* sp. bacterial isolates were able to inhibit the growth of *V. harveyi* bacteria more strongly than *Veillonella* sp. so further testing used the *Acinetobater* sp. bacterial genus. After in vitro testing (Table 4), P3 consisting of *S. cerevisae* and *Acinetobacter* sp. isolates (1:1) had the highest inhibition zone diameter with strong inhibition against *V. harveyi* compared to other treatments, and was significantly different from the control. The mechanism of the inhibition process of *V. harveyi* bacteria is that β-Glucan produces stimulation for phagocytosis activity and is assisted by *Acinetobacter* sp. which can inhibit bacterial growth. According to Hadiuzzaman *et al.* (2022), β-Glucan can stimulate phagocytosis activity. Meanwhile, endophytic bacteria from the genus *Acinetobacter* are able to produce Succinamide Conjugate Diacid (SCD) compounds. Based on research by Wu *et al.* (2011), SCD is a new compound from *Acinetobacter* that can inhibit the growth of several strains of bacteria tested such as *S. aureus, Vibrio, B. subtilis,* and *E. coli*. This shows that SCD can inhibit the growth of

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both Gram positive and Gram negative bacteria and shows that SCD is a broad antibacterial agent. However, the inhibitory activity was still below the positive control (P1). While the treatment of β-Glucan *S. cerevisae* + *Acinetobater* sp. for P4, P5, and P6 have moderate inhibition with values that are not much different, which means that they are still able to inhibit bacterial growth but are not effective treatments.

Table 4. Results of Antibacterial Activity Test of *Acinetobater* sp. Against *V. harveyi*

Treatment	Average Inhibition Zone Diameter (mm)	Category
P1 (Antibiotic)	40.63±0.93	Very strong
P2 (Aquades)	0±0	Not inhibit
P3	14.86±0.63	Strong
P4	9.91±0.8	Moderate
P5	8.40±0.81	Moderate
P6	8.63±0.74	Moderate

Total Hemocyte Count (THC) Calculation

The increase in Total Hemocyte Count (THC) shows the overall number of haemocytes consisting of agranular (hyaline), semi-granular and granular haemocytes. Based on Figure 3, the highest THC value occurred in P3, which is probiotic β-Glucan *S. cerevisae* with endophytic bacteria *S. alba* (1:1). However, it was significantly different from the other shrimp groups after the challenge test. P4, P5, and P6 showed similar results, due to *V. harveyi* bacterial infection. Infection causes an increase in granular cells in pathoge-infected shrimp parts, so that total hemocytes increase, because blood cells will migrate to pathogen-infected areas. Furthemore, the increase in the number of hemocytes in shrimp shows that the phytochemicals such as tannins, flavonoids, and alkaloids and saponins work to activate cellular defense cells by increasing cells that play a role in hemocyte immunity cells which are then able to reduce the number of parasites or infections (Takwin et al., 2022).

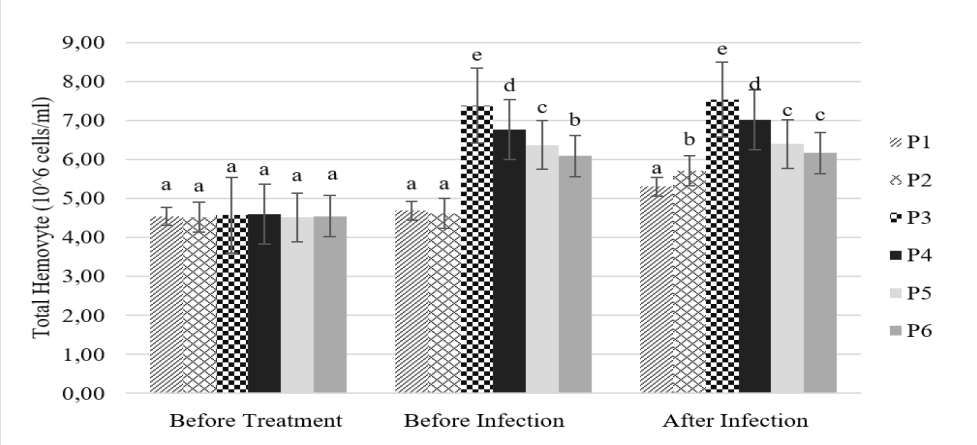

Figure 3. Graph of Total Hemocyte Count Values

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Differential Hemocyte Count (DHC) Calculation

Based on the observation of DHC, the number of granular cells after the challenge test in P6 was 61,11% with a higher value than P2 (positive control) of 56.32%. However, P6 has a value that is not significantly different from P3, where P3 is 60.56%. Then in semi-granular cells, P6 and P2 did not give significantly different results, namely 21.46% and 21.11%. Based on the two types of cell percentages, it shows that granular cells play a large role, but when there are unfavorable conditions, these two types of cells will decrease. Granular cells themselves have a function in the process of producing phenoloxidase enzymes which have an important role in the defense system during pathogen attacks. The semi-granular cells obtained were 16.18-25.23%, still within the normal range of 13-49% (Johansson *et al.*, 2000). While the highest hyaline cells were found in P5 which was 26,34% higher than P2 which was 22.57%. The increase in the number of hyaline cells is related to phagocytic activity (Fadillah *et al.*, 2019). The relationship between hyaline cells and the phagocytosis process is that hyaline cells are responsible for phagocytosing and encapsulating pathogens when foreign bodies enter the shrimp body (Siregar *et al.*, 2024).

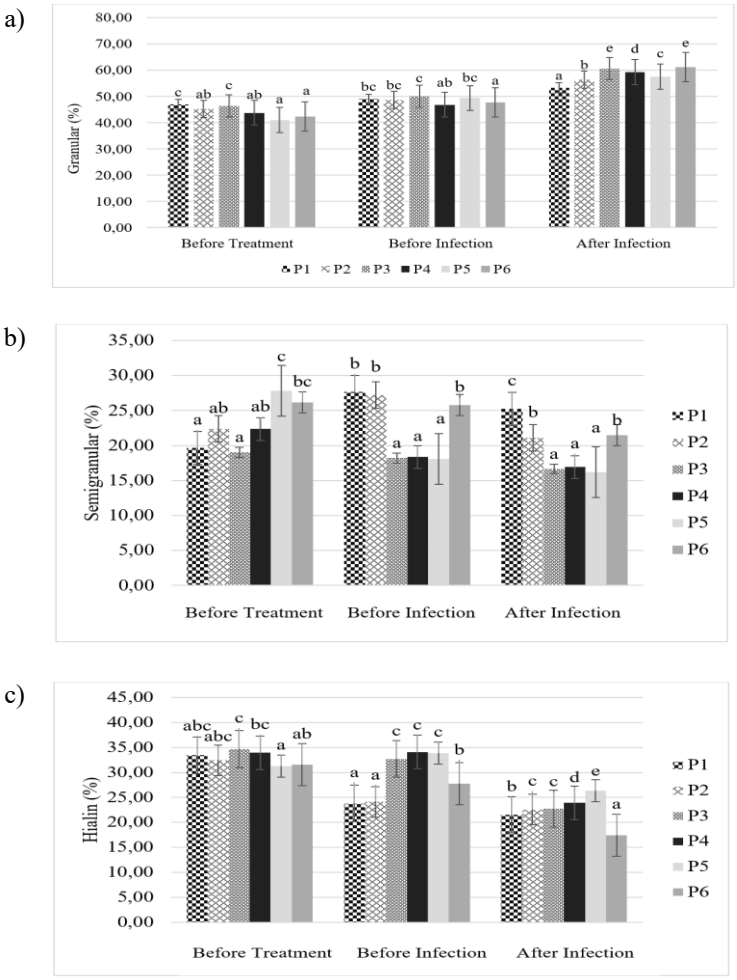

Figure 4. (a) Graph of Granular Cell Percentage (b) Graph of Semi Granular Cell Percentage (c) Graph of Hyaline Cell Percentage.

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Survival Rate

Based on Figure 5, the highest SR value was found in the P1 treatment of 90% as a negative control without *V. harveyi* infection (Fadillah *et al.*, 2019). While the lowest SR value in P2 amounted to 72.5%. The low SR results in P2 prove that infection from *V. harveyi* bacteria during the challenge test process can cause high mortality. In the P3, P4, P5, and P6 treatments, the SR values are 85%, 85%, 82.5% and 75%, respectively. The SR value of all combined feed treatments is higher than the positive control (P2), indicating the effectiveness of the addition of β-Glucan *S. cerevisiae* and the combination of endophytic bacteria *S. alba* leaves on increasing the SR value of *L. vannamei* after a challenge test with *V. harveyi* infection. According to Widigdo (2013), *L. vannamei* survival rate is classified as good as long as the SR value is > 70%.

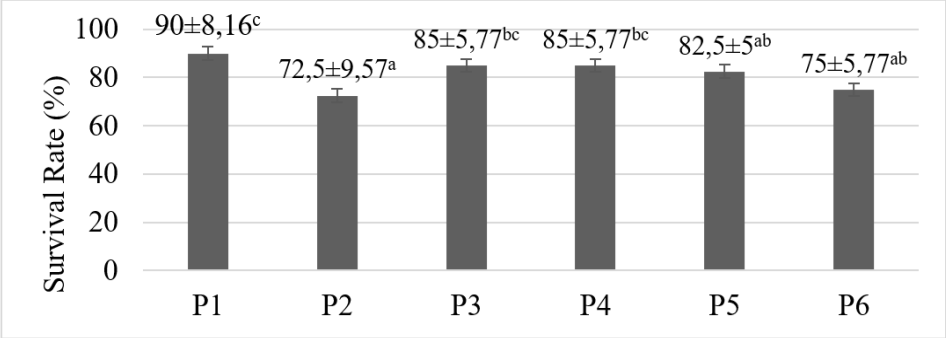
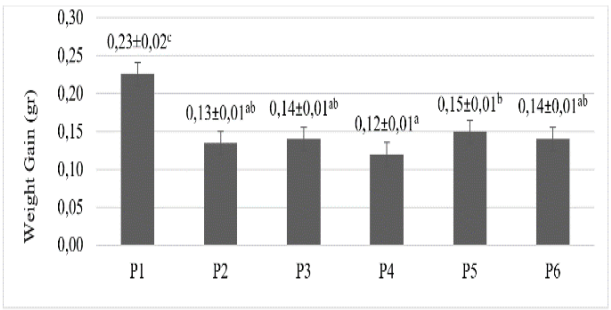


Figure 5. Survival Rate Chart

Determination of Growth Rate

Giving a combination of probiotic feed to *L. vannamei* infected with *V. harveyi* gives the effect of good weight and length growth of *L. vannamei*. Based on Figure 6, *S. cerevisae: S. alba* (2: 1), namely P5 has better growth than P2 (positive control), and the growth values for P3 and P5 are not significantly different. The addition of a combination of β-Glucan *S. cerevisiae* can increase growth, because *S. cerevisiae* contains nucleotides that can increase fish appetite so that growth can increase. In addition, *S. cerevisiae* also improved intestinal morphometry (villi length, villi width, crypt depth, thickness of villi wall) and improved the intestinal microbiota (del Valle *et al.*, 2023).

(a)



(b)

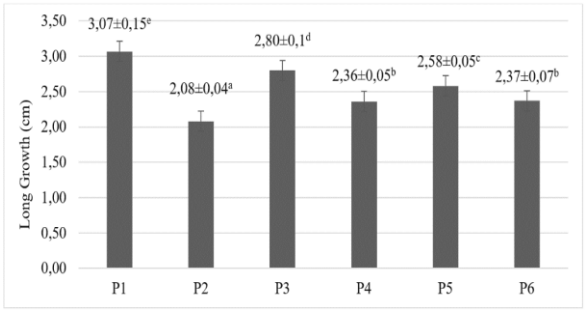


Figure 6. (a) Weight Growth Chart (b) Length Growth Chart

Clinical Symptoms

Clinical symptoms (Figure 7) show that after infection with *V. harveyi*, the tail turns red, the rostrum turns red, and there are

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black spots on the body segment of the cephalothorax. According to Sarjito *et al.* (2012), the clinical symptoms of shrimp attacked by vibriosis in ponds include the characteristics of melanosis on the body, there are white spots, telson and red tail. Morphological changes that occur such as swimming legs (pleopod), reddened telson, necrosis of the tail (uropod), melanosis in the shrimp body segment (Utami, 2016).

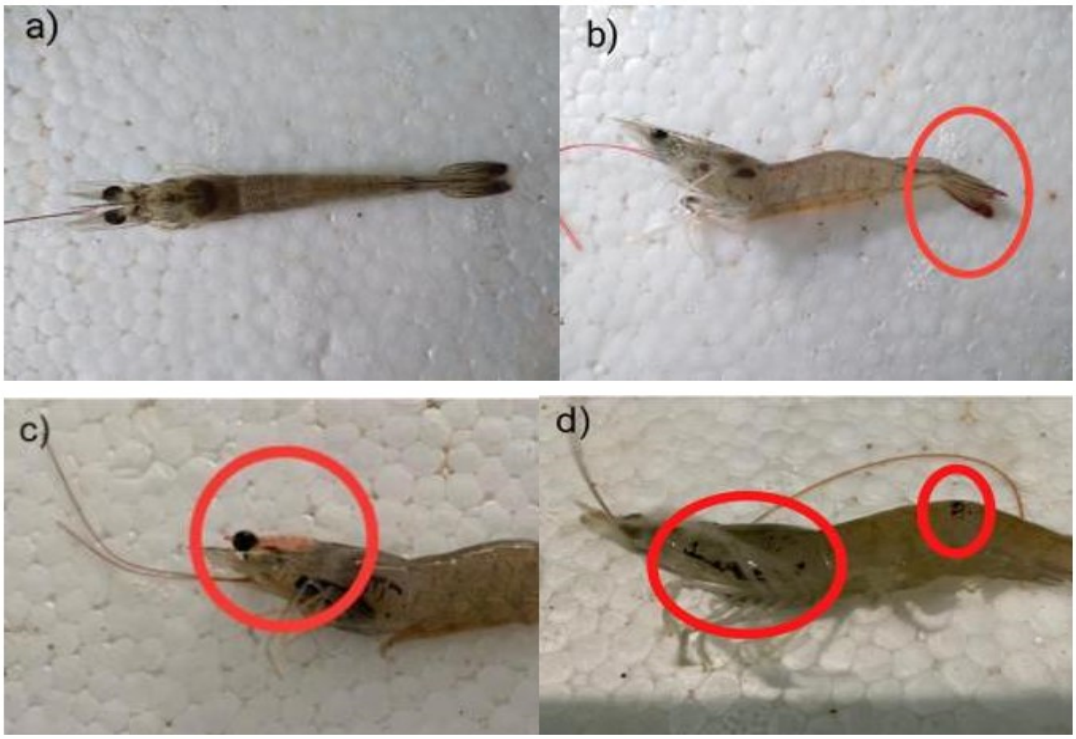

Figure 7. Clinical symptoms of shrimp challenged with *V. harveyi* (a) normal shrimp, (b) red tail, (c) reddened rostrum, (d) black spots on body segments and on the cephalothorax

Water Quality Monitoring

Based on the results of water quality monitoring (Table 5) during treatment showed optimal results for shrimp growth, including parameters: temperature, pH, Dissolve Oxygen (DO), and salinity. All parameter results are within normal standards. The optimal temperature for *L. vannamei* growth is between 26-32 ^{O}C, the optimal pH of shrimp farming is 7.0-8.5, the optimal salinity is 15-35 ppt, and the standard DO for *L. vannamei* is 3-8 ppm (Supriatna *et al.*, 2022; Safitrah *et al.*, 2020). As for the nitrate parameter obtained at 10-50 ppm, it has met the quality standard of <50 ppm. This concentration is not too dangerous for shrimp life, because they can be used to increase the growth of plankton as natural food for shrimp (Awanis *et al.*, 2017). The nitrite ranges from 0.1-0.5 ppm. The nitrite concentration tolerance threshold for shrimp rearing is 0,06-0,42 ppm (Dahlan *et al.*, 2017). The ammonia is in the safe category for *L. vannamei* farming which is below 0.1 ppm. The high content of ammonia will interfere with the process of growth and development of shrimp to cause death (Pasongli & Dirawan, 2015).

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Table 5. Water quality monitoring results

Treatment	Temperature (ºC)	pH	DO (ppm)	Salinity (ppt)	Ammonia (ppm)	Nitrite (ppm)	Nitrate (ppm)
P1	26.1-27	7.6-8.3	5.3-7.5	20	0-0,15	0,1-0,25	10-25
P2	26-27,3	7.9-8.4	5.8-7.7	20	0,15-0,25	0,25-0,5	25-50
P3	26.4-27.7	7.7-8.5	6.1-6.5	20	0-0,15	0,1-0,5	10-50
P4	26.1-27.7	7.4-8.4	5.7-7.4	20	0-0,15	0,1-0,5	10-50
P5	26.2-27	7.5-8.2	5.6-6.8	20	0,15-0,25	0,1-0,5	25-50
P6	26.8-27.8	7.6-8.2	5.7-5.8	20	0,15-0,25	0,25-0,5	25-50
Tolerance	26-32	7.0-8.5	3-8	15-35	<0,1	0,06-0,42	<50

CONCLUSION

Based on the results of the study, it can be concluded that the combination of endophytic bacteria *S. alba* and β-Glucan *S. cerevisiae* as probiotic feed has an influence on the shrimp defense system and survival. The best treatment was P3 with *Total Haemocyte Count* (THC) of 7.54x10^6 cells/ml and *Differential Haemocyte Count* (DHC) (granular 60.56%, semi granulocytes 16.68%, hyaline 22.76%) with survival rate of 85%. The probiotic administration had a significant effect and became the optimum formulation to inhibit the growth of *V. harveyi.*

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AUTHORS'CONTRIBUTIONS

The contribution of each author is as follows, ASA and SM; Conceptualization and drafting of the manuscript. SM and BBT; validation, supervision, and formal analysis. SM, ASA and ST; Collecting and conducting sample evaluation. SM, ASA and ST; Performed statistical analysis and preparation of tables and figures. All authors have read, reviewed and approved the final manuscript.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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REFERENCES

Apriliani, M., & Haditomo, A. H. C. (2016). Keanekaragaman Agensia Penyebab Vibriosis pada Udang Vaname (*Litopenaeus vannamei*) dan Sensitivitasnya terhadap Antibiotik. Journal of Aquaculture Management and Technology, 5(1), 98-107.

Aulia, R. N., & Sulistiyaningsih, R. (2019). Kandungan Metabolit Sekunder Dan Aktivitas Senyawa Bioaktif Tumbuhan Mangrove Perepat (*Sonneratia alba*). Farmaka, 17(3), 151-156.

Awanis, A. A., Prayitno, S. B., & Herawati, V. E. (2017). Kajian kesesuaian lahan tambak udang vaname dengan menggunakan sistem informasi geografis di Desa Wonorejo, Kecamatan Kaliwungu, Kendal, Jawa Tengah.

pISSN 2550-0910; eISSN 2579-4817
Available online at https://e-journal.unair.ac.id/JoAS

Buletin Oseanografi Marina, 6(2), 102-109.

Ayiku, S., Shen, J. F., Tan, B. P., Dong, X. H., & Liu, H. Y. (2020). Effects of dietary yeast culture on shrimp growth, immune response, intestinal health and disease resistance against *V. harveyi*. Fish and Shellfish Immunology, 102, 286-295.

Blaxhall, P. C., & Dalishley. (1973). The Haemothological Assessment of The Health of Fresh Water Fish. A Review of Selected Literature. Journal of Fish Biology, 4, 593-604.

Cempaka, L. (2015). Pengaruh Variasi Kecepatan Agitasi pada Produksi B-Glukan dari *Saccharomyces cerevisiae*. Al-Kauniyah, 8(1), 21-26.

Dahlan, J., Hamzah, M., & Kurnia, A. (2017). Pertumbuhan Udang Vaname (Litopenaeus vannamei) yang dikultur pada sistem bioflok dengan penambahan probiotik. Journal of Fishery Science and Innovation, 1(1), 19-27.

Damayanti, S. S., O. Komala, & E. M. Effendi. (2018). Identifikasi Bakteri dari Pupuk Organik Cair Isi Rumen Sapi. Ekologia: Jurnal Ilmiah Ilmu Dasar dan Lingkungan Hidup, 18(2), 63-71.

Darwantin, K., Sidik, R., & Mahasri, G. (2016). Efisiensi penggunaan imunostimulan dalam pakan terhadap laju pertumbuhan, respon imun dan kelulushidupan udang vannamei (Litopenaeus vannamei). Jurnal Biosains Pascasarjana, 18(2), 123-139.

del Valle, J. C., Bonadero, M. C., & Fernández-Gimenez, A. V. (2023). *Saccharomyces cerevisiae* as probiotic, prebiotic, synbiotic, postbiotics and parabiotics in aquaculture: An overview. Aquaculture, 569, 739342.

Effendie, M. I. I. (1997). Biologi Perikanan. Yayasan Pustaka Nusatama, Yogyakarta.

Evan, Y., Indrawati, A., & Pasaribu, F. H. (2021). Pengembangan uji cepat metode koaglutinasi untuk mendeteksi antigen *Vibrio parahaemolyticus* penyebab penyakit vibriosis pada udang vanamei (*Litopenaeus vannamei*). Biodidaktika: Jurnal Biologi dan Pembelajarannya, 16(1), 45-57

Fadillah, N., Waspodo, S., & Azhar, F. (2019). Penambahan Ekstrak daun mangrove *Rhizophora apiculata* pada pakan udang vaname (*Litopenaeus vannamei*) untuk Pencegahan Vibriosis. Journal of Aquaculture Science, 4(2), 91-101.

Fitri, Z. M., Kismiyati, K., & Mubarak, A. S. (2018). Daya Antibakteri Ekstrak Daun Api-Api (Avicennia alba) terhadap *V. harveyi* Penyebab Vibriosis secara In vitro. Jurnal Ilmiah Perikanan dan Kelautan, 10(2), 131-136.

Hadiuzzaman, M., Moniruzzaman, M., Shahjahan, M., Bai, S. C., Min, T., & Hossain, Z. (2022). β-Glucan: Mode of Action and its Uses in Fish Immunomodulation. Frontiers in Marine Science, 9, 905986.

Harpeni, E., & Saefulloh, A. (2015). Assay Of Acinetobacter As Competitor To Vibriosis Luminescence Bacteria In Pacific Pacific White Shrimp (*Litopenaeus vannamei*). Aquasains, 4(1), 223-328.

Hoar, Y., Salosso, Y., & Santoso, P. 2020. Identifikasi Parasit Dan Bakteri Vibrio Pada Kerang Darah (*Anadara granosa*) di Perairan Tanah Merah, Kecamatan Kupang Tengah. Jurnal Aquatik, 3(2), 57-66.

Ida, N. C., Sariono, H., & Putra, H. E. E. (2022). Metode Penyimpanan Mikroba *Saccharomyces cereviseae* dengan Menggunakan Teknik Cryogenic Freezing di Laboratorium Biosain. Jurnal Pengembangan Potensi Laboratorium, 1(2), 67-72.

Kabatia, M., Khumaira, A., & Bimantara, A. (2023). Isolation and Characterization of Local Microorganisms (MOL) of Bamboo Shoots (*Dendrocalamus asper*) and Its Effect on the Growth of Pakcoy Plants (*Brassica rapa* L.). Bioedukasi, 21(2), 152-165.

KKP. (2022). Kelautan dan Perikanan Dalam Angka Tahun 2022. Jakarta: Pusat Data, Statistik dan Informasi, 86-88.

Lei, H., Li, S., Lu, X., & Ren, Y. (2021). Oral administration of *Saccharomyces cerevisiae* displaying VP28-VP24 confers protection against white spot syndrome virus in shrimp. Virus Research, 302, 198467.

Panab, D. J., Enawati, L. S., & Nenobais, M. (2020). Pengaruh Level Kamir *Saccharomyeces cerevisiae* Sebagai Inokulum dalam Proses Fermentasi Tepung Sabut Kelapa Muda Terhadap Perubahan Kandungan Nutrisi. Jurnal Peternakan Lahan Kering, 2(2), 895-903.

Pardede, M. A., Widanarni, W., Sukenda, S., & Yuhana, M. (2024). Evaluation of Dietary Microencapsulated Synbiotics *Pseudoalteromonas piscicida* 1UB, Bacillus NP5, and Mannan-Oligosaccharides to Prevent

pISSN 2550-0910; eISSN 2579-4817
Available online at https://e-journal.unair.ac.id/JoAS

Vibrio parahaemolyticus Infection in Pacific White Shrimp *Penaeus vannamei.* Aquaculture International, 32(2), 2077-2091.

Pasongli, H., & Dirawan, G. D. (2015). Zonasi Kesesuaian Tambak Untuk Pengembangan Budidaya Udang Vaname (*Penaeus vannamei*) Pada Aspek Kualitas Air Di Desa Todowongi Kecamatan Jailolo Kabupaten Halmahera Barat. Jurnal Bioedukasi, 3(2).

Puspita, D. (2020). Isolasi, identifikasi dan uji produksi yeast yang diisolasi dari nira kelapa. Biosfer: Jurnal Biologi dan Pendidikan Biologi, 5(1), 1-5.

Rachmawati, D., Hutabarat, J., Susilowati, T., Samidjan, I., & Pranggono, H. (2020). Penambahan *Saccharomyces cerevisiae* pada pakan buatan komersial benih lele sangkuriang (*Clarias gariepinus* var. Sangkuriang) terhadap efisiensi pemanfaatan pakan, pertumbuhan, dan kelulushidupan. Jurnal Ilmiah Perikanan dan Kelautan, 19(2).

Rosmiati, R., Tenriulo, A., Nurhidayah, N., Suryati, E., & Parenrengi, A. (2020). Isolasi dan Identifikasi senyawa Aaptaminoid dari *Aaptos aaptos* dan Potensi Pemanfaatannya untuk Pencegahan infeksi bakteri *V. harveyi*. Jurnal Riset Akuakultur, 15(1), 41-50.

Safitrah, L., Setyowati, D. N. A., & Astriana, B. H. (2020). Efektivitas ekstrak kulit pisang kepok (*Musa balbisiana* Colla) untuk menurunkan kanibalisme pada udang Vaname (*Litopenaeus vannamei*). Jurnal Kelautan, 13(1), 36-44.

Seniati, S., Marbiah, M., & Irham, A. (2019). Pengukuran Kepadatan Bakteri *V. harveyi* Secara Cepat Dengan Menggunakan Spectrofotometer. Agrokompleks, 19(2), 12-19.

Siregar, K. A., Sitepu, G. S. B., & Fain, H. (2024). Analisis Granular Dan Hialin Pada Udang Kaki Putih (*Litopenaeus vannamei*) Dengan Pemberian Asam Lemak DHA (Docosahexaenoic acid). Journal of Tropical Marine Science, 7(1), 25-30.

Suleman, S. (2020). Ekstrak Polisakarida Rumput Laut *Ulva lactuta* Sebagai Imunostimulan Untuk Melawan *V. harveyi* pada Udang Vaname *(Litopenaeus vannamei*). Prosiding Seminar Nasional IPPEMAS, 1(1), 675-683.

Sulistiyani, T. R., & Lisdiyanti, P. (2016). Keragaman bakteri endofit pada tanaman *Curcuma heyneana* dan potensinya dalam menambat nitrogen. Widyariset, 2(2), 106-117.

Supriatna, M., Mahmudi, M., & Musa, M. (2020). Model pH dan hubungannya dengan parameter kualitas air pada tambak intensif udang vaname *(Litopenaeus vannamei*) di Banyuwangi Jawa Timur. Journal of Fisheries and Marine Research, 4(3), 368-374.

Suryani, S., & A'yun, Q. (2022). Isolasi Bakteri Endofit dari Mangrove *Sonneratia alba* Asal Pondok 2 Pantai Harapan Jaya Muara Gembong, Bekasi. Jurnal Ilmiah Biologi, 1(2), 12-18.

Syafitri, E., Afriani, D. T., Siregar, B., & Gustiawan, Y. (2021). Kandungan Fitokimia dan Uji Aktivitas Antibakteria Ekstrak Daun Mangrove (*Sonneratia alba*) Secara Invitro Terhadap *Aeromonas Hydrophila*. Jurnal Riset Akuakultur, 15(4), 253-259.

Takwin, B. A., Nurâ, D., & Azhar, F. (2022). Pengaruh pemberian ekstrak daun jarak pagar (*Jatropa curcas* L.) terhadap sistem imun udang vaname (*Litopenaeus vannamei*) yang diinfeksi bakteri *V. harveyi*. Jurnal Ilmu Kelautan Kepulauan, 5(2), 598-609.

Utami, W. (2016). Pengaruh Salinitas Terhadap Efek Infeksi V. harveyi Pada Udang Vaname (*Litopenaeus vannamei*). Journal of Aquaculture Management and Technology, 5(1), 82-90.

Widigdo, B. 2013. Bertambak Udang dengan Teknologi Biocrete. Kompas, Jakarta.

Wu, D., Chang, H., Wang, Y., & Xin, M. (2011). Isolation, Structure Determination And Antibacterial Activities Of Succinamide Conjugate Diacid From *Acinetobacter* sp. BJ-L. Microbiological Research, 166(3), 155-160.

Yanti, D., Rahmawati, R., & Kurniatuhadi, R. (2021). Karakteristik Morfologis dan Fisiologis Bakteri Endofit dari Akar Napas Tumbuhan *Avicennia marina* (fork) vierh di Mempawah Mangrove Park. Biologica Samudra, 3(2), 166-183.