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Coelomocyte activity and pathogenicity description of nypa palm worm (*Namalycastis rhodochorde*) after injection with *Aeromonas* sp. NrBF9 isolated from fecal pellets of nypa palm worm

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Abstract. *Yanti AH, Kurniatuhadi R, Setyawati TR, Ramadani R. 2022. Coelomocyte activity and pathogenicity description of nypa palm worm (*Namalycastis rhodochorde*) after injection with* Aeromonas *sp. NrBF9 isolated from fecal pellets of nypa palm worm. Biodiversitas 23: 5439-5445.* Coelomocytes in the Annelida group play a role in the immune system by recognizing and destroying foreign objects that enter the body. A study of the character and type of nypa palm worm coelomocytes (Polychaetes) can provide an overview of nypa palm worm resistance to pathogenic microorganisms. Thus researchers can determine the resistance of worms during cultivation based on developmental stages. This study aimed to determine the total and differential coelomocytes of nypa palm worms induced by *Aeromonas* sp. NrBF9, and to describe the pathogenicity of *Aeromonas* sp. NrBF9 against palm worms. Observation of the total number of coelomocytes, differential coelomocytes, and character of coelomocytes was carried out after injecting pathogenic bacteria directly into the body of the nypa palm worm. The bacterial test suspension was divided into three treatments, namely P1 (2.7×10^7 cells/mL of *Aeromonas* sp. NrBF9), P2 (3.2×10^5 cells/mL of *Aeromonas* sp. NrBF9), and P3 (3.4×10^3 cells/mL of *Aeromonas* sp. NrBF9). Non-induced worms were used as controls. The treated worms were then incubated for 72 hours. The total number of coelomocytes was calculated using the cubicle count method, whereas differential coelomocytes using the smear method. The pathogenicity of the nypa palm worms was described descriptively by determining the morphology of the nypa palm worms after 72 hours of bacterial injection. The results showed an increase in the total number of coelomocytes of nypa palm worms in all injection treatments with *Aeromonas* sp. NrBF9. The highest total coelomocyte count was obtained in treatment P1(10-3) of 70.58 x 10^3 cells/mm^3. The number of differential coelomocytes also increased, especially in type II and IV-amoebocyte cells. The description of disease resistance and symptoms of nypa palm worms after being induced by *Aeromonas* sp. NrBF9 in the form of sores and discoloration of the internodes near the injection site became white and healed on the third day. The description of the type and character of coelomocytes and the resistance of nypa palm worms to pathogens are expected to be the basis for understanding worm cultivation activities in the future.

Keywords: *Aeromonas* sp. NrBF9, coelomocytes, immune response, nypa palm worms

INTRODUCTION

Nypa palm worms (*Namalycastis rhodochorde*) have been used by people in West Kalimantan, Indonesia, as bait for fishing for fish and shrimp traditionally. In addition, nypa palm worms are also used as feed for local shrimp culture. Junardi et al. (2019) have developed and utilized nypa palm worms as an ingredient in pellet flour for fish feed. The potential of nypa palm worms as aquaculture feed is due to the protein content of fish pellets from nypa palm worms reaching 76%. However, the fulfillment of the demand for nypa palm worms currently still depends on nature. Direct exploitation can cause damage to the microhabitat and decrease the nypa palm worm population in its natural habitat. Therefore, the development of nypa palm worm cultivation needs to be carried out to overcome the decline in the nypa palm worm population. Until now, there has been no massive and controlled cultivation. The success of nypa palm worm cultivation in the laboratory is influenced by fertilization and the mass production of larvae and young worms (Junardi et al. 2014). Currently, the cultivation of nypa palm worms is still experiencing obstacles in slow growth and pathogen attack. According to Junardi and Riyandi (2020), when cultured in the laboratory for nypa palm worms, it takes 3-4 months to reach 40 segments, and the survival rate is still low. The death of nypa palm worms is caused by protozoan attacks and diseases caused by bacteria and fungi.

If the number exceeds the standard limit, indigenous pathogenic bacteria found in the digestive tract can cause disease in nypa palm worms. Setyawati's research regarding screening and testing for pathogenicity on nypa palm worms obtained NrBF9 bacterial isolates, which were suspected to be indigenous pathogenic bacteria from fecal pellets of nypa palm worms (Setyawati et al. 2020). The isolates were suspected to be pathogenic bacteria with hemolysis ability based on the test on blood agar. Pathogenic bacteria such as *Aeromonas hydrophila* can produce various toxins, namely exotoxins in the form of hemolysin, cytotoxin, enterotoxin, and endotoxin in the form of lipopolysaccharide (Rozy et al. 2018). Hemolytic toxins can lyse erythrocytes and leukocytes and cause

tissue necrosis resulting in anemia and lesions in infected sites. The pathogenicity test used a bacterial suspension of NrBF9 isolate induced into the nypa palm worm, causing lesions on the body surface of the nypa palm worm, presumably due to the ability of the bacteria to hemolyze and triggering the severance of the nypa palm worm body to death (Setyawati et al. 2020). The nypa palm worm's defense system against disease or bacterial infection is influenced by cellular defense mechanisms. An understanding of the type of defense cell and the mechanism of immunity of nypa palm worms has an urgency to be known in the context of its potential in aquaculture development. As an estuary polychaete worm that has the potential to develop aquaculture, the type and mechanism of immunity are the basis for the development and increase in production as well as a model for studying its eco-immunological aspects and strategies. Cuvillier-Hot et al. (2014) stated that a polychaete group is a group of potential annelids as a learning model and strategy in developing the eco-immunology of marine and estuarine organisms.

Annelids, especially the polychaete worm group, have defense mechanisms mediated by cellular and humoral responses. Coelomocytes carry out the coelomocytes response of the annelid group in the form of amoebocytes with phagocytic activity, which freely circulates in the coelomic cavity and can enter tissues and organs (Bodo et al. 2021). In comparison, the humoral response as an aspect of immunity is mediated by macromolecules in the extracellular fluid, such as secreted antibodies, complement proteins, and specific antimicrobial peptides (Wu et al. 2020). The coelomic fluid contains coelomocytes which play a role in fighting pathogens and helping to cope with stress against environmental changes (Maltseva et al. 2014). Several studies have stated that coelomocytes from the coelomic cavity function as an immune system in the form of phagocytosis (Karetin 2021) and stress response and fertility (Park et al. 2015). Research on the character of coelomocytes has been carried out by several researchers on various invertebrate animals, such as Japanese sea cucumber *Aposthicopus japonicus* (Taguchi et al. 2016), *Echinaster brasiliensis* (Asteroidea), *Holothuria tubulosa* (Holothuroidea), *Eucidaris tribuloides*, *Arbacia lixula*, *Lytechinus variegatus*, and *Echinometra funnynter* (Echinoidea) (Jose 2021). However, scientific information regarding the profile and ability of coelomocytes in the nypa palm worm to defend against pathogens cellularly is not yet known. Previous research (Setyawati et al. 2020) has been carried out based on the defense response of nypa palm worms that have entered the mature phase directly. However, the types and descriptions of coelomocytes have not been described after being injected with pathogenic bacteria.

Based on this report, it is essential to conduct further research on coelomocyte activity against pathogenic bacteria through analysis of total and differential coelomocytes as well as an overview of pathogenicity in coelomic fluid based on test parameters of a various number of cell treatments by *Aeromonas* sp. NrBF9 isolated from fecal pellets of nypa palm worms after injecting into nypa palm worms. This study is the basis for developing a strategy to increase the immunity of nypa palm worms which will be used as eco-immunology-based cultivation worms according to the character of life in their natural habitat.

MATERIALS AND METHODS

Materials

The materials used included the sub-mature phase of nypa palm worms, *Aeromonas* sp. NrBF9 from Setyawati collection (2020), MC Farland 0.5 standard solution, Turk solution, Leishman dye solution, NaCl, Nutrient Agar (NA).

Methods

Research design

The experimental design was Completely Randomized Design (CRD) which consisted of 5 treatments, P1, P2, P3, and six replications. The experimental design used was: (i) Control: Nypa palm worms were not induced by *Aeromonas* sp. NrBF9; (ii) P1: Nypa palm worms are induced by 2.7×10^7 cells/mL of *Aeromonas* sp. NrBF9; (iii) P2: Nypa palm worms are induced by 3.2×10^5 cells/mL of *Aeromonas* sp. NrBF9; (iv) P3: Nypa palm worms are induced by 3.4×10^3 cells/mL of *Aeromonas* sp. NrBF9. The parameters measured were the total number of coelomocytes and the number of differential cells from the coelomic fluid of nypa palm worms. The description and description of coelomocyte type and condition of nypa palm worms after injection of *Aeromonas* sp. NrBF9.

Bacterial suspension preparation

Preparation of *Aeromonas* sp. NrBF9 suspension using physiological NaCl solution (NaCl 0.9%) following the previous method (Setyawati et al. 2020). The bacterial suspension was adjusted to the turbidity standard of the spectrophotometer suspension with a wavelength of 600 nm to reach an absorbance of 0.132, equivalent to a turbidity standard of 0.5 Mc Farland suspension (10^8 CFU/mL). Bacterial dilution was carried out in stages until the lowest dilution was 10^8 as a stock culture. Nypa palm worms acclimatized for ten days would be injected with *Aeromonas* sp. NrBF9 based on a 0.5 Mc Farland dilution into 10^8, 10^7, 10^6 cells/mL and control (not induced by bacterial isolates).

Nypa palm worm preparation

The animal test used were male nypa palm worms in the sub-mature phase. The sub-mature phase of nypa palm worms is known by microscopically observing the coelomic fluid before the study. A 0.3 mL of coelomic fluid was taken, then blotted on the surface of the glass slide and observed with a microscope with a magnification of 1000 times. The sub-mature phase is characterized by the presence of early gamete cells (20-50 μm in diameter) in the coelomic fluid smear.

All worms are used with a body length of 90-100 cm, a body weight of 20-25 grams, and several segments among

698-769. The worms were acclimatized for seven days in prepared containers and tubs. Maintenance of worms is determined by spraying water, so the soil does not dry out. After the injection, the nypa palm worms were kept. Observations were carried out by counting the total number of coelomocytes using the cubicle counting method at 72 hours following Homa (2018), differential coelomocytes using the smear method that had been stained with Leishman's dye, and descriptive descriptions of the pathogenicity of nypa palm worms.

Coelomocyte preparation

The coelomic fluid of nypa palm worms was taken as much as 0.3 mL following Arredondo et al. (2014). The collection was carried out using a method developed by the nypa palm worm research team. The coelomic fluid sampling method uses a capillary tube that is burned in the middle above the Bunsen burner and then pulled in the opposite direction. The result is two capillary tubes that are pointed and still have cavities. Sampling is determined by injecting a tapered capillary tube between the segments of the nypa palm worm. Then the coelomic fluid is collected and stored in a microtube. Furthermore, the preparation of coelomocyte counts can be carried out.

Total coelomocyte calculation

Coelomocytes in a collected coelomic fluid were counted manually using a hemocytometer. The collected coelomocytes were taken using a leukocyte Thoma pipette to a scale of 0.5. Furthermore, Turk's diluent solution was taken up on a scale of 11. The coelomocytes in the Thoma pipette were homogenized by shaking the pipette slowly. Furthermore, the coelomocytes were pipetted into a hemocytometer to be observed under a microscope with a magnification of 400 times and then counted using a tally counter. Calculation of coelomocytes by adopting the calculation of leukocytes in vertebrate animals in the following formula:

$$\sum L = \frac{NI \times P}{0,4}$$

Where, ∑L: Total coelomocytes; NI: number of coelomocytes found in 64 boxes counted; P: Dilution (20); 0.4: Total volume of coelomocytes in 64 counted boxes.

Coelomocyte differential calculation

Coelomocyte differential calculations were carried out using the smear method with Leishman staining using the modification of the Taguchi et al. (2016) method. Coelomocyte differential counting was carried out by observing smear preparations that had been stained with Leishman stain. The smear preparations that have been made are dried for 5 minutes. Leishman dye was dripped until it pooled on the object glass for 1 minute. Distillation liquid in distilled water is added in a ratio of 2:1 for 10-12 minutes. The distilled liquid was discarded, then the smear preparation was dried (HIMedia 2019). The smear preparations that had been stained were examined using a microscope with 1000 magnification. Previously the preparations had been dripped with immersion oil. The cells found were counted as 200, and recorded the number of coelomocyte types was counted. Coelomocyte count by adopting differential leukocyte cell count in vertebrate animals.

Data analysis

Coelomocyte's total and coelomocyte differential data were analyzed using one-way Analysis of Variance (ANOVA) with a level of 5%. If it is significantly different, it is continued with Duncan's test with a level of 0.05. Morphological changes of infected nypa palm worms are shown descriptively.

RESULTS AND DISCUSSION

Polychaete has developed several defense mechanisms that efficiently recognize and eliminate foreign materials, microbes, or parasites. In general, coelomocytes in annelids can be divided into two main types: amoebocytes and eleocytes (Vetvicka and Sima 2009; Correira et al. 2021). The type and profile of the number of nypa palm worm (*Namalycastis rhodochorde*) coelomocytes have never been characterized. Either standard numbers without attacked pathogens, after exposure to pathogens, as well as of coelomocytes changes due to pathogenic stress. However, it is expected that the type and description of the number of nypa palm worm coelomocytes have the same value as other genera of Polychaete, as described in *Ophelia limacina* (Belova and Zhadan 2011), *Amphitrite johnstoni* and Nereid Polichaete by Dales in 1963, and *Eurythoe complanata* (Arredondo et al. 2014). The coelomocytes of nypa palm worms showed increasing in the total number of coelomocytes. It indicates that the coelomocytes defend against local pathogenic bacteria induced by *Aeromonas* sp. NrBF9. Coelomocyte activity can be seen from the average total coelomocyte of normal nypa palm worms. After being induced by *Aeromonas* sp. NrBF9, there is a difference in the increase in total coelomocytes counts (Table 1). Coelomocytes in worms will increase due to interference with microorganisms and environmental changes. It follows Irizar's (2015) research, which stated that *Eisenia fetida* exposed to metal in vitro showed premature eleocyte death and was followed by an increase in amoebocyte complexity.

The total coelomocytes increased as in treatment P1, which was 70.58×10^3 cells/mm^3, treatment P2 was 61.51×10^3 cells/mm^3, and treatment P3 was 53.11×10^3 cells/mm^3. While the control did not experience an increase of 36.45×10^3 cells/mm^3. The highest increase in the number of nypa palm worm coelomocytes after being induced by *Aeromonas* sp. NrBF9 occurred in treatment P1 for 72 hours.

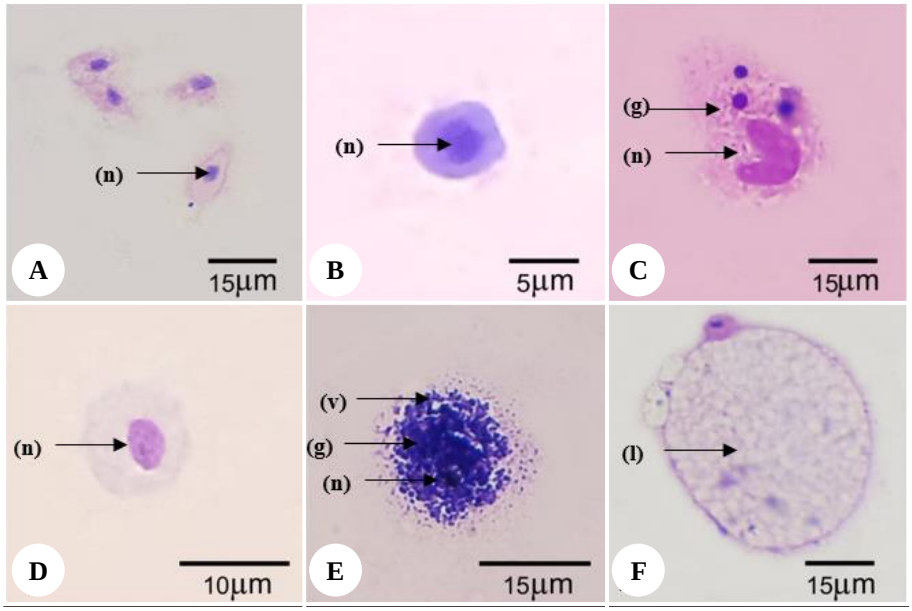

Figure 1. Amoebocyte-Coelomocyte and eleocyte morphology of *Namalycastis rhodochorde* after injection by *Aeromonas* sp. NrBF9. Description: A. Type I; B. Type II; C. Type III; D. Type IV; E. Type V; F. eleocytes; g. granules; l. fat drops; n. nucleus; v. vacuole

Table 1. Average results of total coelomocytes of nypa palm worms before and after injection of *Aeromonas* sp. NrBF9 with different numbers of the cell

Treatments	Average total coelomocytes (10^3 cell/mm^3)		Coelomocyte difference (10^3 cell/mm^3)
	Before	After	
Control	38.50 ± 5.10^a	36.45 ± 7.30^a	- 2.05 ± 4.94^d
P1	37.64 ± 4.18^a	70.58 ± 3.73^b	+ 32.94 ± 1.83^a
P2	37.71 ± 3.93^a	61.51 ± 6.01^c	+ 23.80 ± 7.64^b
P3	38.55 ± 5.11^a	53.11 ± 5.92^d	+ 14.55 ± 8.98^c

Note: +: increase; -: decrease

Table 2. Average results of differential coelomocytes of nypa palm worms before and after induction of *Aeromonas* sp. NrBF9 with different numbers of cells

	Types	Treatments			
		Control	P1	P2	P3
Before (%)	Type I	36.75 ± 2.25^a	38.00 ± 2.39^a	39.08 ± 3.28^a	38.17 ± 0.88^a
	Type II	3.50 ± 0.95^a	4.50 ± 0.90^a	10.00 ± 4.15^a	9.83 ± 3.60^a
	Type III	14.00 ± 3.27^a	13.42 ± 1.07^a	22.67 ± 4.18^a	25.83 ± 4.80^a
	TypeIV	11.17 ± 2.94^a	10.42 ± 1.53^a	21.17 ± 6.94^a	20.00 ± 5.06^a
	Type V	4.25 ± 0.93^a	4.17 ± 1.17^a	8.33 ± 1.86^a	9.00 ± 2.76^a
	Eleosit	30.33 ± 3.08^a	29.50 ± 2.37^a	29.83 ± 3.54^a	29.50 ± 2.37^a
After (%)	Type I	36.50 ± 1.55^b	27.75 ± 1.47^a	26.67 ± 1.99^a	27.75 ± 2.21^a
	Type II	3.83 ± 0.52^a	15.08 ± 0.97^c	13.67 ± 0.97^b	14.33 ± 0.93^{bc}
	Type III	14.17 ± 0.98^b	2.17 ± 0.61^a	1.42 ± 0.97^a	1.25 ± 0.69^a
	TypeIV	11.42 ± 0.66^a	30.00 ± 1.00^b	33.83 ± 1.75^c	33.33 ± 1.63^c
	Type V	4.83 ± 0.93^b	1.25 ± 0.524^a	1.42 ± 0.38^a	1.83 ± 0.51^a
	Eleosit	29.25 ± 1.33^c	23.75 ± 2.16^b	23.00 ± 2.05^{ab}	21.50 ± 1.14^a
Difference (%)	Type I	-0.25 ± 3.57^a	-10.25 ± 3.57^b	-12.42 ± 4.05^b	-10.42 ± 2.46^b
	Type II	+0.33 ± 0.81^c	+10.58 ± 0.97^a	+8.67 ± 1.83^b	+9.46 ± 2.04^{ab}
	Type III	+0.17 ± 3.88^a	-11.25 ± 0.88^b	-9.92 ± 2.29^b	-11.67 ± 2.44^b
	TypeIV	+0.25 ± 2.77^b	+19.58 ± 1.28^a	+23.25 ± 4.62^a	+23.33 ± 2.25^a
	Type V	+0.58 ± 1.24^a	-2.92 ± 1.24^b	-2.75 ± 0.82^b	-2.67 ± 1.08^b
	Eleosit	-1.08 ± 3.48^a	-5.75 ± 4.30^{ab}	-6.83 ± 4.75^b	-16.00 ± 6.51^b

Note: +: increase, -: decrease

The increase in nypa palm worm coelomocytes followed the number of cells of bacterial injection. The higher bacteria cell number affected the increase in the total coelomocytes number. The increasing number of total coelomocytes was in response to the nypa palm worm's immune cells because it is infected with bacteria and causes inflammation. Coelomocytes perform complex cellular mechanisms to engulf or ingest foreign particles, also known as phagocytosis. It follows the statement of Homa (2018) that the ability of phagocytosis plays an essential role in the host's immune response to bacterial infection.

Nypa palm worms have a cellular defense mechanism mediated by coelomocytes in the form of amoebocytes. Based on the results of the study, there were two types of coelomocytes in the coelomic fluid of nypa palm worms, namely amoebocytes and eleocytes (Figure 1). In general, coelomocytes in annelids can be divided into two main types, namely amoebocytes and eleocytes, amoebocytes can be of several types (Vetvicka and Sima 2009; Homa 2018). The amoebocytes observed in nypa palm worm's coelomocytes were typed I amoebocytes, type II amoebocytes, type III amoebocytes, type IV amoebocytes, and type V amoebocytes (Figure 1A-1E). The study results on nypa palm worms after being induced by *Aeromonas* sp. NrBF9 showed that there was an increase and decrease in the mean differential of coelomocytes from several types of amoebocytes (Table 2).

The study results on nypa palm worms after being induced by *Aeromonas* sp. NrBF9 showed that there was an increase and decrease in the mean differential of coelomocytes from several types of amoebocytes (Table 2). An increase occurred in type II and type IV amoebocytes. Both types of amoebocytes are macrophage cells that are active in phagocytosis and encapsulation of bacteria. This is following Cooper's research in 1992 that type II coelomocytes can encapsulate, which is then destroyed by coelomocytes. Encapsulation is the process of wrapping an unknown foreign material. The counts of type II and IV-like amoebocytes basophils obtained in the first extrusion were similar to those reported by Toupin and Lamoureux (1976). Basophilic cells have been identified as the most immunoreactive coelomocytes (Arredondo et al. 2014).

Decrease in the number of coelomocytes and several types of amoebocytes in nypa palm worms that *Aeromonas* sp. NrBF9 has induced, namely type I, type III, type V, and eleocytes. Porchet's study in 1987 showed that type I coelomocytes are characterized by the presence of microfilament form. It sometimes curls around the nucleus, which can encapsulate parasites quickly in less than one hour. The results of Cuvillier's research (Cuvillier et al. 2014), G3 as type III amoebocytes act as NK cells. Type V amoebocytes are also active in phagocytosis. But because of their large cell size, these cells can phagocytize significant microorganisms. It can be assumed that type V amoebocytes are active against significant parasitic infections. The ability of eleocytes to play a role in defense is most likely limited. As stated by Schenk et al. (2016), eleocytes play a role in egg nutrition, excretion, and osmotic balance in the maturation stage. However, according to Nesto et al. (2018), eleocytes are associated with the maturation of oocytes that provide nutrition for growing eggs and in some Nereids.

The results of the pathogenicity test of *Aeromonas* sp. NrBF9 against nypa palm worms in vivo did not cause death in nypa palm worms, although the first 72 hours showed infection in the form of wounds on the skin surface. However, the next day the regeneration process occurred and the wound on the surface of the segment began to return to normal. Based on the results of the post-challenge mortality calculation showed an average survival rate of 100% in all treatments.

Based on the results of the post-challenge mortality calculation showed an average survival rate of 100% in all treatments. Bacterial infection with increased doses can lead to infection of the worms resulting in illness and death (Setyawati et al. 2020). It is presumed that the bacteria injected into the nypa palm worm have not reached the appropriate density lethal dose because the treatment was carried out using a multilevel dilution of the bacterial suspension. Differences in the level of maturation also affect the appearance of disease symptoms in the nypa palm worm. Sub-mature worms used in this study showed a recovery process after four days of exposure to bacteria. It is different from the research by Setyawati et al. (2020) using nypa palm worms with mature conditions. It indicates that the phase associated with gonad maturity can affect the body's defense system against pathogens. Tests on immature gonads (sub-mature) in this study are to see the description of the coelomocyte response and whether pathogenic bacteria can kill nypa palm worms. The assumption of previous researchers stated that worms in the sub-mature phase had the character of early gamete cells that were starting to develop. It causes the energy of the nypa palm worms in the sub-mature phase not to focus on the development of gamete cells so that they still have an excellent immune response. Baranzini et al. (2020) stated that the restoration or healing of wound segments and the immune response in the annelid group was influenced by food availability and developmental stages. Developmental stages have an essential role in looking at the pattern of humoral and cellular immune system responses so that it can be seen how critical points are in the management and handling of nypa palm worm aquaculture in the event of a pathogen attack at each phase or developmental stage.

Table 3. Results of calculated survival rate of nypa palm worms before and after induction of *Aeromonas* sp. NrBF9 with different numbers of cells

Treatments	∑ Number of worms on day 0 (tails)	∑ Number of worms on day 7 (tails)	The survival rate of worms (%)
Control	6	6	100
P1	6	6	100
P2	6	6	100
P3	6	6	100

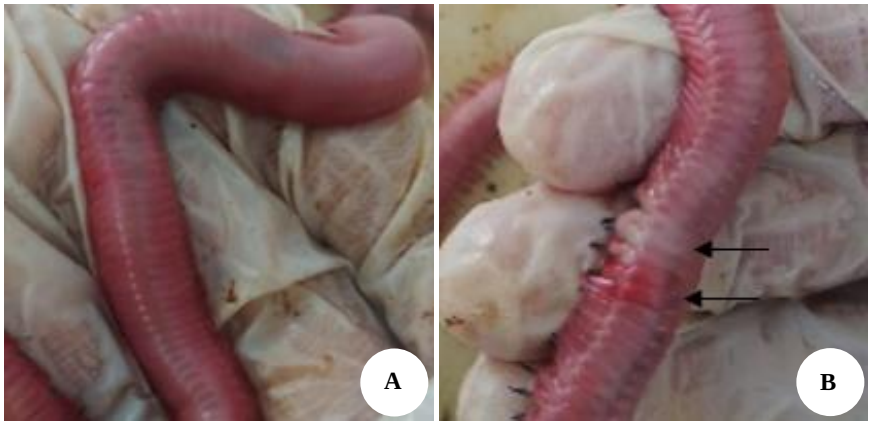

Figure 2. Morphology of the nypa palm worm (*Namalycastis rhodochorde*). Description: arrows indicate lesions. A. Before; B. After induced by *Aeromonas* sp. NrBF9

Nypa palm worms are worms with a single life cycle until gonad maturity. Maturity is thought to cause nypa worms to focus on gamete maturation, making them more susceptible to pathogen attack. It is suspected that immature worms can defend the body against the bacteria that have been injected. However, based on the appearance of the body morphology of the nypa palm worm after *Aeromonas* sp. NrBF9 induced it, there was a change in the color of the segment turning white in the nypa palm worm body near the injection area and other areas (Figure 2). Coelomocytes are thought to play an essential role in the blastema regeneration process and restoration of damaged segments. Bodo et al. (2021) stated that granular amoebocytes-coelomocytes cooperate with eleocytes in regenerating wounds or segmenting lesions in earthworms. This mechanism is thought to also occur in nypa palm worms. Therefore, further research is needed on the specific function of coelomocytes in wound repair due to pathogenic infections.

Meanwhile, in the control treatment, there were no body segments that turned white (Figure 2A). Some parts of the infected nypa palm worm's segment show a redder or darker color. Prochazcova et al. (2019) stated that changes in body color in earthworms as a body defense activity by the formation of brown fluid in the coelomic cavity caused by coelomic aggression around foreign cells that interfere with such bacteria and other aggregates. Changes in the color of the morphology of the nypa palm worms around the injection area also disappeared after the 5th to 7th days. The recovery of worms from abnormal conditions and environmental stress can be recovered within a week, marked by the coelomic fluid returning to normal as in *N. rhodochorde*. The pattern of the total number of coelomocytes contributes to the development nypa palm worms into aquaculture products. The acceleration of recovery of immature nypa palm worms also provides an idea in the utilization of nypa palm worm's coelom fluid as an antibacterial product of pathogens in aquaculture.

In conclusion, we found the defense ability of nypa palm worms against *Aeromonas* sp. NrBF9 isolates with an increase in the total number of coelomocytes from the normal number. The highest increase in the number of total coelomocytes from the P1 (2.7×10^7 cells/mL) treatment which was 70.58×10^3 cells/mm^3 was greater in number than the control, which was 36.45×10^3 cells/mm^3 and the differential mechanism of coelomocytes in nypa palm worms also changed in number with increasing type II and type IV-amoebocytes to respond of the worm's body's defenses against pathogenic bacteria. The pathogenicity of *Aeromonas* sp. NrBF9 did not occur in each treatment because the lethal dose was not achieved in sub-mature nypa palm worms. Although at 72 hours after injection, symptoms of infection in the form of sores or lesions and discoloration of the nypa palm worms were seen, on the next day, the nypa palm worms began to heal and were healthy like worms without treatment. It is the basis for researchers to research specific immune responses at each developmental stage to support the cultivation of nypa palm worms.

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