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ARTICLE

GROWTH OF NYPA PALM WORMS (*Namalycastis rhodochorde*) JUVENILES FED ON NYPA PALM FRONDS FERMENTED BY CELLULOLYTIC BACTERIA NrLtC4 AND NrLtG2

[*Pertumbuhan Juvenil Cacing Nipah (Namalycastis rhodochorde) yang diberi Pakan Pelepah Nipah terfermentasi Bakteri Selulolitik NrLtC4 dan NrLtG2*]

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ABSRACT

Nypa palm worms (*Namalycastis rhodochorde*: Polychaetes) play important ecological roles as detritivores of organic matter in mangrove ecosystems, bioindicators of water pollution, and sediment stabilizers in estuarine environments. *N. rhodochorde* also shows potential for use in fermented feed-based aquaculture due to its high nutritional value as a natural food source for aquatic organisms. Cellulolytic lactic acid bacteria isolated from the coelomic fluid and gastrointestinal tract of *N. rhodochorde*, identified as isolates NrLtC4 and NrLtG2, have potential as starter cultures for fermenting niypa palm fronds, which are used as feed in nypa palm worm cultivation. This study aimed to evaluate the growth of juvenile *N. rhodochorde* fed nypa palm fronds fermented by the cellulolytic bacteria NrLtC4 and NrLtG2. Juveniles were maintained under consistent stocking density, container size, and water volume, with three treatments arranged in a completely randomized design: unfermented nypa palm fronds (control), nypa palm fronds fermented with isolate NrLtC4, and nypa palm fronds fermented with isolate NrLtG2. The results demonstrated that fermented feed significantly influenced both the number of body segments [$p = 0.00021$ and 0.0028 ($p < 0.05$)] and body length [$p = 0.0020$ and 0.0035 ($p < 0.05$)] of the juveniles. Juveniles fed nypa palm fronds fermented with strain NrLtC4 showed increases of 29.6% in segment number and 58.5% in body length, while those fed fronds fermented with strain NrLtG2 exhibited increases of 36.6% and 63.9%, respectively. The highest juvenile survival rate was observed in the NrLtC4 fermented feed group at 72%, followed by 69.33% in the NrLtG2 group. These findings suggest that bacterial isolates NrLtC4 and NrLtG2 have promising potential as a consortium feed to enhance the growth of nypa palm worms in aquaculture.

Keywords: cellulolytic bacteria, feed fermentation, growth, juveniles *Namalycastis rhodochorde*, nypa palm frond

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ABSTRAK

Cacing nipah (Namalycastis rhodochorde: Polychaetes) memiliki fungsi ekologis sebagai detritivor bahan organik dalam ekosistem mangrove, bioindikator pencemaran air dan agen stabilisasator sedimen lingkungan estuari. N. rhodochorde berpotensi untuk dikembangkan secara akuakultur berbasis pakan fermentasi karena memiliki kandungan nutrisi yang baik untuk sumber pakan alami biota air. Bakteri asam laktat selulolitik yang diisolasi dari cairan selom dan saluran gastrointestinal N. rhodochorde dengan kode isolat NrLtC4 dan NrLtG2 berpotensi sebagai starter fermentasi pelepah nipah yang dibutuhkan sebagai pakan dalam budi daya cacing nipah. Tujuan penelitian ini untuk mengetahui pertumbuhan juvenil N. rhodochorde yang diberi pakan pelepah nipah terfermentasi bakteri selulolitik NrLtC4 dan NrLtG2. Juvenil dipelihara dengan padat tebar, ukuran wadah pemeliharaan dan volume air yang sama yang terdiri dari tiga perlakuan menggunakan rancangan acak lengkap. Perlakuan yang diberikan adalah pemberian pelepah nipah tanpa fermentasi (kontrol), pemberian pelepah nipah terfermentasi isolat NrLtC4, dan isolat NrLtG2. Hasil penelitian menunjukkan bahwa perlakuan pakan fermentasi berpengaruh nyata terhadap jumlah segmen [p = 0.00021 dan 0.0028 (p < 0.05)] dan panjang tubuh [p = 0.0020 dan 0.0035 (p < 0.05)] mutlak dan spesifik dari juvenil. Juvenil yang diberi perlakuan pakan fermentasi oleh strain NrLtC4 menunjukkan peningkatan jumlah segmen sebesar 29,6% dan panjang tubuh 58,5%, sedangkan pakan fermentasi strain NrLtG2 terjadi peningkatan masing-masing sebesar 36,6% dan 63,9%. Tingkat kelangsungan hidup juvenil tertinggi tercatat pada perlakuan pakan yang difermentasi bakteri NrLtC4 yaitu sebesar 72%, disusul perlakuan pakan dengan bakteri NrLtG2 sebesar 69,33%. Isolat bakteri NrLtC4 dan NrLtG2 berpotensi dikembangkan sebagai pakan konsorsium yang meningkatkan pertumbuhan cacing nipah di akuakultur.

Kata kunci*: bakteri selulolitik, fermentasi pakan, juvenil Namalycastis rhodochorde, pelepah nipah, pertumbuhan*

INTRODUCTION

Nypa palm worms (*Namalycastis rhodochorde*) are polychaete annelids inhabiting West Kalimantan's mangrove forests. They play a crucial ecological role as detritivores by decomposing organic matter and enhancing nutrient cycling (Junardi *et al*., 2020; Miri *et al*., 2023; Yanti *et al*., 2025). Additionally, *N. rhodochorde* serve as bioindicators of water pollution and help stabilize sediments, improving estuarine environmental quality (Putro *et al*., 2025). In biomedicine, these worms show promise due to bioactive compounds with antimicrobial and anti-inflammatory properties, potentially aiding wound healing (Ghazaly *et al*., 2024). Moreover, their adaptability and high nutritional value make them a sustainable alternative feed in aquaculture, reducing dependence on costly commercial feeds (Junardi, 2021; Kurniatuhadi *et al*., 2025; Setyawati *et al*., 2021).

The local community in the Sungai Kakap Subdistrict depends on *N. rhodochorde* for their livelihoods, owing to its significant market value (Yanti *et al*., 2019). Containing over 58% protein, this species represents a high-quality food source for aquaculture purposes (Boyd *et al*., 2022; Junardi, 2021). Nevertheless, the demand within the aquaculture industry currently surpasses the supply available from wild populations.

N. rhodochorde employs a monotelic reproductive strategy characterized by the external release of mature gametes and body segment fragmentation, facilitating gradual population growth (Junardi *et al*., 2010). Laboratory investigations indicate that the developmental period from the larval stage to the 40-segment juvenile stage spans approximately three to four months (Junardi & Riyandi, 2020). Suboptimal feed formulations hinder juvenile growth (Lall & Dumas, 2022). Junardi & Riyandi (2020) identified fermented nypa palm fronds treated with EM-4, a commercial biofertilizer used in agriculture, as the most effective feed for promoting the growth of *N. rhodochorde*. Nevertheless, the potential application of cellulolytic bacterial consortia and their capacity to degrade other organic compounds derived from habitat substrates and bacteria associated with the digestive tract of the nypa palm worm remain unexplored.

The use of nypa palm fronds as animal feed is limited by their low nutritional value, mainly due to high fiber content, especially cellulose (Suryadi *et al*., 2021). Fermentation with lactic acid bacteria (LAB) can improve their quality by promoting cellulose degradation. Yanti *et al*. (2020) isolated ten LAB strains from fecal pellets, coelomic fluid, and the gastrointestinal tract of *N.*

rhodochorde. In vitro studies showed that isolates NrLtC4 and NrLtG2 have high cellulolytic and proteolytic activity, making them promising starter cultures for fermenting nypa palm fronds. Kurniatuhadi *et al*. (2025) reported that NrLtC4 and NrLtG2 decomposed nypa frond cellulose by 16.67–23.26% and 20.14–26.39%, respectively. These isolates are well-suited for processing nypa frond waste, a natural food source for juvenile nypa palm worms, supporting their cultivation. Further research is needed to assess the effectiveness of feed produced from fermented nypa palm fronds using these isolates. This bacterial consortium, derived from the nypa worm's habitat and digestive tract, could replace EM-4 as a fermentation and biodegradation agent for palm fronds.

The objective of this study was to assess the growth of juvenile *N. rhodochorde* when fed nypa palm fronds fermented by cellulolytic bacteria, specifically NrLtC4 and NrLtG2. The results of this research are expected to offer valuable insights into feed selection for the cultivation of *N. rhodochorde*, thereby enhancing its survival and growth to address the increasing demand for *N. rhodochorde* as a feed source in the aquaculture industry.

MATERIALS AND METHODS

Bacterial Isolate Preparation

The NrLtC4 and NrLtG2 strains were isolated from the intestinal and digestive tract fluids of *N. rhodochorde*, as reported by Yanti *et al*. (2020). Prior to isolate preparation, a revived isolates cultured for fermentation process was conducted. This process was performed aseptically, beginning with the sterilization of all tools and materials, particularly the carboxymethyl cellulose (CMC) media. Sterilization was achieved using an autoclave at 121 °C and 0.15 MPa for 15 minutes. Subsequently, the NrLtC4 and NrLtG2 strains were recultured in carboxymethyl cellulose broth (composed of 5 g CMC, 1.36 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 5 g $MgSO_4 \cdot 7H_2O$, 2 g NaCl, and 1 g yeast extract) for further analysis.

Feed Fermentation Process

The feed manufacturing process comprises two primary stages: starter preparation and the fermentation of nipa fronds. All procedures are performed under aseptic conditions. Initially, the starter is prepared by formulating a carboxymethyl cellulose (CMC) broth, which serves as the starter medium. The CMC medium is sterilized by autoclaving at 121°C and 0.15 MPa for 15 minutes. Subsequently, the revived isolates designated for fermentation are inoculated into 50 ml of the CMC medium and incubated with agitation at 150 revolutions per minute (rpm) for 18 hours at ambient temperature (Kurniatuhadi *et al*., 2025). Following incubation, the optical density (OD) was measured at 660 nm, and an inoculum exhibiting an OD of 0.5—corresponding to the exponential growth phase—is considered suitable for use as the fermentation starter in nypa palm frond feed production. The fermentation process involves combining 950 ml of distilled water, 1 kg of wet nypa palm fronds, and 50 ml of the prepared fermentation starter in sterile fermentors. This mixture was subjected to anaerobic fermentation by thorough mixing and subsequent incubation in a fermentation vessel at ambient temperature for 30 days.

Initial Rearing of Larvae

The fertilization of nypa palm worms was performed in vitro by combining mature oocytes and spermatozoa within a 1000 ml glass jar. A magnetic stirrer operating at 30 rpm was employed to facilitate the process (Salsabila *et al*., 2024). The resulting fertilized embryos were subsequently transferred into a containers containing 50 liters of water with a salinity of 13 parts per thousand (ppt). These containers were maintained by providing an initial feed of the microalga *Chlorella vulgaris* until the larvae developed six juvenile chaetigers. On the tenth day post-fertilization, larvae exhibiting the six-chaetiger stage, indicative of the juvenile phase, were identified through microscopic examination and transferred from the initial maintenance container to the test feeding container.

Feed Experiments

This study employed an experimental approach utilizing a completely randomized design (CRD) comprising three treatments with five replications each. The treatments included unfermented nypa palm fronds, nypa palm fronds fermented with the NrLtC4 isolate, and nypa palm fronds fermented with the NrLtG2 isolate. The experiment was conducted in containers measuring 41 × 32 × 14 cm, each filled with 3 liters of water at a salinity of 13 ppt and containing 10 grams of the respective fermented nypa palm fronds. Ten-day-old larvae at the 6-chaetiger stage were collected using a plastic pipette and enumerated. Each rearing tank housed 30 juvenile *N. rhodochorde* individuals (Edo *et al*., 2020).

Feeding was provided only at the onset of juvenile rearing and was not resumed until the end of the observation period. The nypa palm worms were reared for 60 days until they reached the immature stage. Growth parameters, including segment gain, body length gain, and survival rate, were recorded at 30-day intervals throughout the rearing period. Water quality parameters—such as temperature (°C), pH, salinity (%), and dissolved oxygen (mg/L)—were measured daily over the 60-day period in each rearing container (Edo *et al*., 2020).

Growth and Survival

The number of segments was quantified by counting the setigers from the thoracic chaetiger to the anal cirri under a microscope. Juvenile body length was measured by extending the juvenile worms on millimeter paper and subsequently measuring the distance from the tip of the prostomium to the pygidium using a tape measure. Relative growth in both segment number and body length was evaluated. Increases in segment number and body length were recorded as absolute and specific gains. The absolute gain in segment number and body length was calculated using the equation $Pt = St - S0$, where St represents the segment number or length at time t, and S0 represents the initial measurement. The specific gain in segment number and body length was determined relative to the rearing duration using the equation $LPS = 100\% \times (St - S0) / t$. Juvenile survival was calculated using the formula $S = 100\% \times (Nt / N0)$, where Nt denotes the number of juveniles at the final observation and N0 represents the number of juveniles at the initial observation (Junardi & Riyandi, 2020).

Data Analysis

The growth response was subjected to statistical analysis utilizing Analysis of Variance (ANOVA) at a significance level of 5%, employing R-Studio version 4.3.2. Subsequent to the ANOVA, Duncan's Test was conducted, also at a significance level of 5%.

RESULTS

Growth

During the rearing process, juveniles of *N. rhodochorde* exhibited growth marked by an increase in both the number of segments and body length. Figure 1 illustrates the growth in the number of segments of juveniles at 0, 30, and 60 days across various treatments. Juvenile nypa palm worms fed fermented nypa palm fronds grew faster and showed a greater increase in the number of segments compared to those fed unfermented fronds.

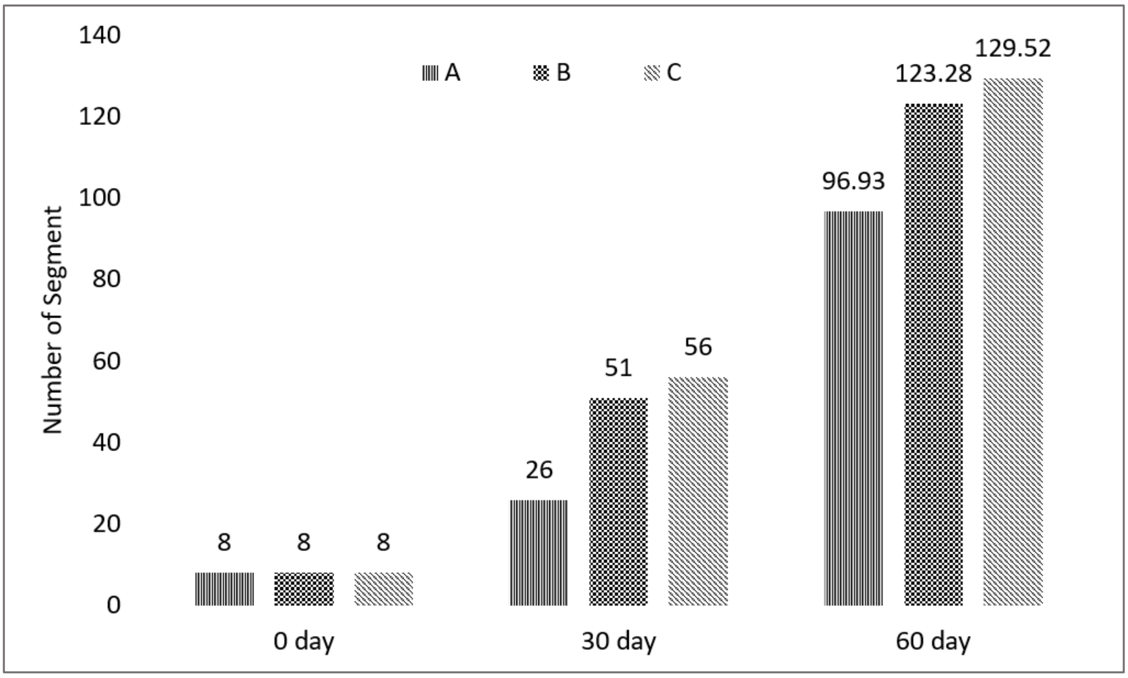

Figure 1. Number of juvenile segments from (A) unfermented nypa palm fronds, (B) fermented nypa frond isolate NrLtC4, and (C) fermented nypa frond isolate NrLtG2 (*Jumlah segmen juvenil pada (A) Pelepah nipah tidak difermentasi, (B) Pelepah nipah terfermentasi NrLtC4, dan (C) Pelepah nipah terfermentasi NrLtG2*).

Figure 2 shows the body length of juveniles at 0, 30, and 60 days across different treatments. Similar to the number of segments, juvenile nypa palm worms fed fermented nypa palm fronds grew in length faster than those fed unfermented fronds.

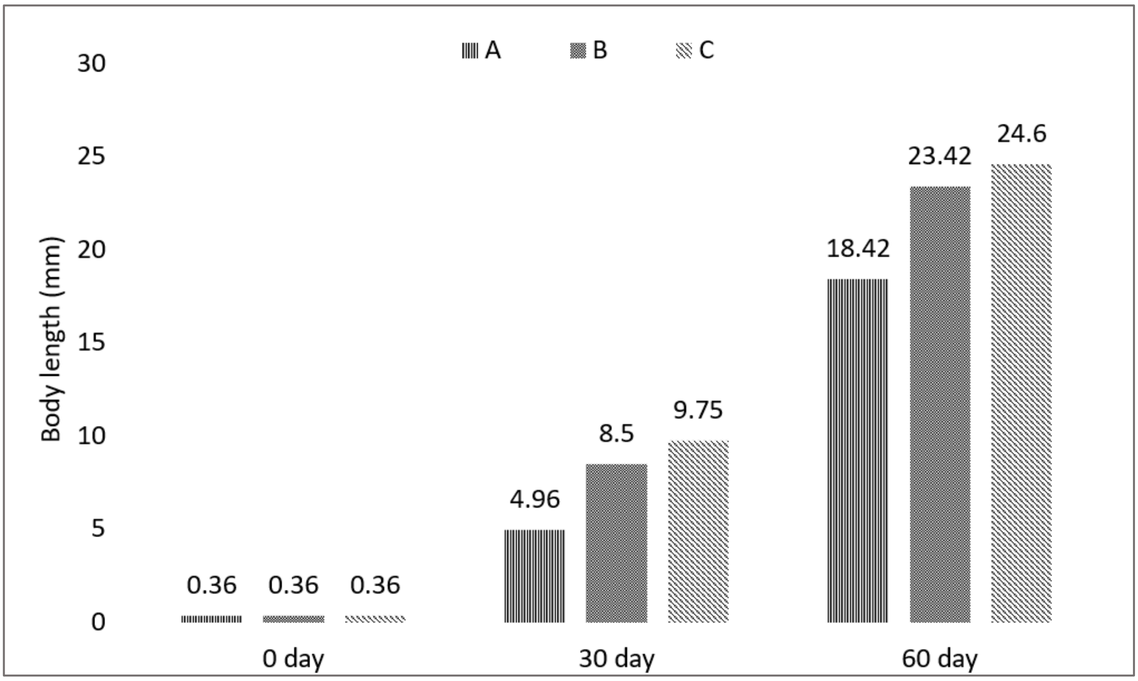

Figure 2. Juvenile body length: (A) unfermented Nypa palm fronds, (B) fermented Nypa palm fronds isolate NrLtC4, (C) fermented Nypa palm fronds isolate NrLtG2 (*Panjang tubuh juvenil pada (A) Pelepah nipah tidak difermentasi, (B) Pelepah nipah terfermentasi NrLtC4, dan (C) Pelepah nipah terfermentasi NrLtG2*).

Juveniles of *N. rhodochorde* utilized at the onset of rearing exhibited eight segments and measured 0.36 mm in body length (Figure 3). These eight-segment juveniles were distinguished by the presence of six chaetigers, which indicated the development of parapodia on six segments beginning from the third segment.

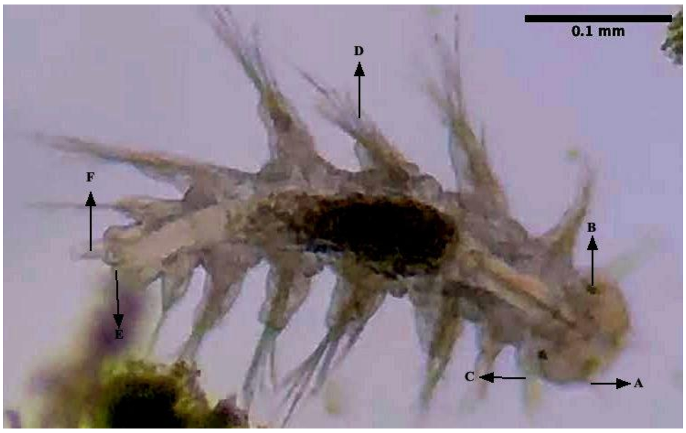

Figure 3. Morphology of 6-chaetiger juveniles, (A) antennae, (B) eyes, (C) tentacle cirri, (D) parapodia, (E) anus, and (F) anal cirri (*Morfologi juvenil 6-setiger, (A) antena, (B) mata, (C) tentakel cirri, (D) parapodia, (E) anus, (F) cirri anal*).

Figure 4. Visual differences in juveniles at day 30 and day 60 across treatments. Bars: 1 mm. (A) unfermented nypa palm fronds, (B) fermented nypa palm fronds isolate NrLtC4, (C) fermented nypa palm fronds isolate NrLtG2 (*Perbedaan visual pada juvenil hari ke-30 dan hari ke-60 di antara perlakuan. Bar: 1 mm. A) Pelepah nipah tidak difermentasi, (B) Pelepah nipah terfermentasi NrLtC4, dan (C) Pelepah nipah terfermentasi NrLtG2*).

During the rearing process, juveniles of *N. rhodochorde* exhibited growth characterized by an increase in both the number of segments and body length. After 30 days of maintenance, the juveniles remained microscopic and could not be observed directly. However, by 60 days, the juveniles had achieved a body length ranging from 18 to 24 mm, rendering them sufficiently large for direct observation. The highest number of absolute and specific segments at the conclusion of the rearing period was observed in treatment C (Table 1). The results of the analysis of variance (ANOVA) for absolute and specific segment gain indicated $p = 0.00021$ ($p < 0.05$) and $p = 0.0028$ ($p < 0.05$), respectively, demonstrating significant differences among the treatments.

Table 1. Absolute and Specific Segmental Gain of Juveniles measurements in body length of *N. rhodochorde* following treatment with nypa palm frond fermentation mediated by bacteria from the nypa palm worm digestive tract (*Pengukuran peningkatan segmental absolut dan spesifik pada Panjang tubuh juvenil N. rhodochorde setelah perlakuan dengan fermentasi pelepah nipah yang dimediasi oleh bakteri dari saluran pencernaan N. rhodochorde*).

Treatment (*Perlakuan*)	**Average Number of Segments** (*Rerata pertambahan jumlah segmen*)	
	Absolute (*Mutlak*)	**Specific** (*Spesifik*) **(%)**
A (Control)	88,93±15,03[a]	4,13±0,26[a]
B (NrLtC4)	115,28±10,34[b]	4,55±0,14[b]
C (NrLtG2)	121,52±9,74[b]	4,63±0,13[b]

The highest absolute and specific body length gain was observed in treatment C (Table 2). The results of the analysis of variance (ANOVA) for absolute and specific body length gain indicated $p = 0.0020$ ($p < 0.05$) and $p = 0.0035$ ($p < 0.05$), respectively, demonstrating significant differences between treatments.

Table 2. Absolute and specific growth measurements in body length of *N. rhodochorde* following treatment with nypa palm frond fermentation mediated by bacteria from the nypa palm worm digestive tract (*Pengukuran pertumbuhan absolut dan spesifik pada panjang tubuh N. rhodochorde setelah perlakuan dengan fermentasi pelepah nipah yang dimediasi oleh bakteri dari saluran pencernaan N. rhodochorde*).

Treatment (*Perlakuan*)	**Average Body Length Growth** (*Rerata Pertumbuhan Panjang Tubuh*)	
	Absolute (*Mutlak*)	**Specific (%)** (*Spesifik*)
A (Control)	18.61±2.95[a]	$66\times10^{-1}\pm26\times10^{-2}$ [a]
B (NrLtC4)	29.5±6.58[b]	$73\times10^{-1}\pm35\times10^{-2}$ [b]
C (NrLtG2)	30.5±6.82[b]	$74\times10^{-1}\pm36\times10^{-2}$ [b]

Correlation Between the Number of Segments and the Body Length

The analysis revealed a positive correlation between the number of segments and the body length of juvenile *N. rhodochorde*, with a correlation coefficient of $R^2 = 0.75$. This value, which approaches 1, suggests that an increase in the number of segments is associated with an increase in juvenile body length.

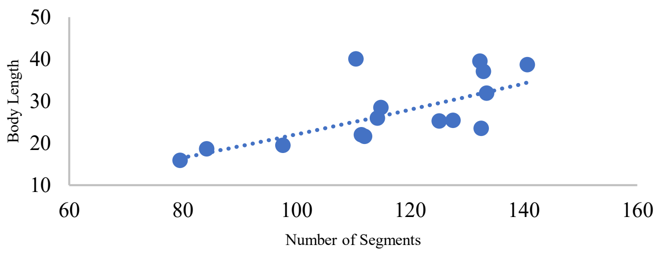

Figure 4. Correlation between body length and segment count in juvenile *N. rhodochorde* demonstrates a positive relationship, indicating that the number of segments increases proportionally with body length. This correlation is associated with the administration of feed fermented using indigenous bacteria of the nypa palm worm (NrLtC4 and NrLtG2) (*Korelasi panjang tubuh dan jumlah segmen juvenil N. rhodochorde. Korelasi positif memperlihatkan jumlah segmen selaras dengan peningkatan panjang tubuh. Keselarasan ini berkaitan dengan penggunaan pakan yang difermentasi dengan bakteri indigenus cacing nipah (NrLtC4 dan NrLtG2*).

Survival Rate

The highest juvenile survival rate was recorded in treatment B, which exhibited a survival rate of 72%, followed by treatment C, with a survival rate of 69.33%. The analysis of variance (ANOVA) conducted on the survival rates yielded a p-value of 0.65 ($p > 0.05$), indicating that there is no statistically significant difference among the treatments

Table 3. Survival rates of *N. rhodochorde* following treatment with nypa palm frond fermentation mediated by bacteria from the nypa palm worm digestive tract (*Tingkat kelangsungan hidup N. rhodochorde setelah perlakuan dengan fermentasi pelepah nipah yang dimediasi oleh bakteri dari saluran pencernaan N. rhodochorde*).

Treatment (*Perlakuan*)	**Survival (%)** (*Sintasan*)
A (Control)	$63{,}33 \pm 4{,}64^{a}$
B (NrLtC4)	$72 \pm 4{,}45^{a}$
C (NrLtG2)	$69{,}33 \pm 4{,}15^{a}$

Environmental Parameters for Rearing Process

Environmental parameters for rearing *N. rhodochorde* juveniles were consistently monitored. Salinity of the cultivation water was maintained at 13 parts per thousand (ppt).

Table 4. Condition and quality of the juvenile rearing media for *N. rhodochorde* during maintenance in each treatment tank were assessed (*Kondisi dan kualitas media pemeliharaan juvenil N. rhodochorde selama pemeliharaan di setiap tangki perlakuan dinilai*).

Parameters (*Parameter*)	**Treatment** (*Perlakuan*)		
	A	**B**	**C**
pH	6,9 - 8,3	6,8 - 8	6,9 - 8,28
	7,59±0,31	**7,53**±0,02	**7,62**±0,05
Temperature	25,3 - 29,5	25 - 29,4	25,2 - 29,5
(*Suhu*) **(°C)**	**27,7**±0,04	**27,63**±0,03	**27,64**±0,04
DO (mg/L)	3,8 - 7,87	4,0 - 8,0	3,8 – 8
	6,04±0,24	**6,19**±0,16	**5,91**±0,21
Salinity (*Salinitas*) **(ppt)**	**13**	**13**	**13**

DISCUSSION

One of the key factors influencing the growth of juvenile *N. rhodochorde* is the type of feed provided. Different types of feed result in varying growth responses in juvenile *N. rhodochorde*. This species is typically found around nypa plants, especially near the roots and in deeper soil layers (Junardi & Setyawati, 2023). Such microhabitats are closely associated with the natural feeding habits of *N. rhodochorde*, which functions as a deposit feeder. Fermented feed aligns well with the species' natural diet, as it contains simpler organic compounds that are more readily assimilated.

The fermentation of nypa palm fronds to enhance the visibility of nypa palm worm growth in aquaculture has been investigated using EM-4 as a biodegradation and fermentation agent. The findings demonstrated an increase in the survival rate of nypa palm worm larvae when fed with feed fermented by an EM-4 consortium (Junardi & Riyandi, 2020). However, the survival and growth parameters, particularly body size and the number of segments, of juvenile nypa palm worms (*N. rhodochorde*) have not been extensively studied, especially in relation to the use of indigenous nypa palm worm bacteria with enzymatic activity.

This study shows that fermenting nypa palm fronds with cellulolytic bacteria (NrLtC4 and NrLtG2) enhances the growth of *N. rhodochorde* juveniles. This aligns with previous research on fermented plant-based feeds for aquatic species. For instance, Neves *et al*. (2024) found that fermenting plant-based feeds with *Lactobacillus acidophilus* improved the nutritional profile, survival, and gut health of *Nile tilapia*. Similarly, fermented lignocellulose-based kitchen waste using *Bacillus licheniformis* and *Yarrowia lipolytica* increased growth and feed efficiency in yellow catfish compared to non-fermented controls (Li *et al*., 2025).

These studies show that microbial fermentation of lignocellulose-rich substrates (crude fiber, cellulose, hemicellulose) breaks down complex structures, increasing the availability of nutrients like proteins, lipids, and soluble carbohydrates. For instance, fermentation of kitchen waste with mixed cultures reduced crude fiber and carbohydrates while increasing soluble amino acids and crude protein. In this study, cellulolytic bacteria such as NrLtC4 and NrLtG2 likely accelerate cellulose and fiber degradation, releasing simple sugars, oligomers, and nutrients trapped in the lignocellulosic matrix (Li *et al*., 2025)

Feeding fermented palm fronds inoculated with two bacterial isolates derived from the digestive tract of nypa palm worms (*N. rhodochorde*) produced results that differed significantly from the control. Data analysis revealed that the increases in segment number and body length observed in treatment C (NrLtG2) were not significantly different from those in treatment B (NrLtC4); however, both treatments exhibited significant differences compared to treatment A (control), which served as the control (Tables 1 and 2). Treatment A consisted of fresh nypa palm fronds, characterized by a complex chemical composition including cellulose, hemicellulose, pectin, and lignin (Wijana *et al*., 2023). Rahman *et al*. (2024) reported that nypa palm plants possess a high fiber content and low fat levels, factors that may constrain their ability to support the growth of *N. rhodochorde* body tissues.

Treatments B and C utilize nypa palm fronds that have been fermented by cellulolytic bacteria through the process of lactic acid fermentation, which facilitates the degradation of cellulose (Jayus *et al*. 2019). This fermentation process generates by-products in the form of carbon dioxide (CO_2) and water (H_2O), resulting in the breakdown of cellulose, a polysaccharide that constitutes a component of crude fiber, into oligosaccharides or monosaccharides. As a result, the concentration of saccharides (carbohydrates) diminishes, which in turn leads to an increase in the percentage of crude protein. The crude protein present in the feed can provide energy during mitotic cell division (Yu *et al*. 2020), thereby promoting an increase in body tissue and facilitating the growth of *N. rhodochorde* segments.

The bacterial isolate NrLtC4 utilized in treatment B belongs to the genus *Lactobacillus* (Yanti *et al*. 2020). In contrast, the bacterial isolate NrLtG2 employed in treatment C is identified as *Bacillus paramycoides* (Setyawati *et al*. 2021). Both *Lactobacillus* and *Bacillus* genera are frequently incorporated as probiotic agents in aquaculture feed due to their ability to inhibit the colonization of gastrointestinal pathogens. Intestinal colonization is essential for probiotics to influence the gut microbiota. Key factors in evaluating the colonization potential of probiotics encompass their capacity to produce exopolysaccharides, adhere to intestinal surfaces, self-aggregate, and inhibit pathogenic microorganisms (Sarita *et al*., 2024). This inhibitory effect is associated with enhanced production and quality in the aquaculture of *N. rhodochorde* (Chaves *et al*. 2017; Khalid *et al*. 2021).

The most significant growth in juvenile specimens was observed in those administered the *Bacillus paramycoides* isolate (NrLtG2), a bacterium known for its ability to degrade organic matter by hydrolyzing starch in mangrove litter through the secretion of the enzyme amylase (Putri *et al*. 2021). *B. paramycoides* is classified as a gram-positive bacterium that possesses the capability to form endospores (Yi *et al*., 2017). Test results indicate that NrLtG2 exhibits tolerance to 0.3% bile acid, suggesting that treatment C may also serve as a probiotic for *N. rhodochorde*, as the presence of endospores confers thermal stability and resistance to gastric acid (Setyawati *et al*. 2021). *Bacillus* species have been documented as probiotic agents within the field of aquaculture (Chau *et al*. 2021). Furthermore, additional research has shown that *Bacillus* species can modulate the microbial composition of the hindgut in *Cherax cainii* and promote specific growth in *Labeo rohita* (Foysal *et al*. 2020; Yousuf *et al*. 2023).

The highest juvenile survival rate was recorded in treatment B, which exhibited a survival rate of 72%, followed by treatment C, with a survival rate of 69.33% (Table 3). Wibowo *et al*. (2022) noted that the survival rate surpassed 50%, suggesting that the type of feed provided is effectively and efficiently digested, thereby supporting juvenile survival. The analysis of variance (ANOVA) conducted on the survival rates yielded a p-value of 0.65 ($p > 0.05$), indicating that there is no statistically significant difference among the treatments. Fermented or unfermented nypa front feed is adequate to fulfill the survival requirements of juvenile organisms. The natural dietary preference of *N. rhodochorde*, which functions as a deposit feeder, consists of organic matter originating from the decomposition of nypa plants. Consequently, all juveniles that were provided with fermented feed demonstrated higher survival rates compared to those in the control group (Junardi, 2021; Junardi *et al*, 2020).

The findings of the study indicate that juvenile mortality was prevalent across all treatment groups. Notably, high survival rates ranging from 70% to 100% were recorded for the polychaete species *Perinereis cultrifera* at a stocking density of 15 individuals within a 40x60 cm rearing container (Elayaraja *et al*., 2011). Conversely, the present study revealed that a stocking density of 30 juveniles resulted in mortality rates of 63.33% in treatment A and 69.33% in treatment C. This underscores the detrimental effects of increased competition for space and resources on juvenile survival. Furthermore, survival rates are influenced by a combination of internal and external factors. Internal factors include body weight, sex, age, movement, acclimation, and oxygen consumption (Rosa & Saastamoinen, 2017). External factors pertain to abiotic conditions, such as water quality and media maintenance (Ramee *et al*., 2020). Additionally, the presence of pathogenic bacteria during the juvenile rearing process can significantly reduce survival rates, particularly in scenarios where water replacement is not conducted throughout the rearing period. Salinity levels were periodically adjusted to maintain a stable level of 13 ppt by adding distilled water when necessary (Table 4). As reported by Junardi and Riyandi (2020), *N. rhodochorde* can thrive at an average salinity of 12 ppt. Edo *et al*. (2020) reported that a suitable salinity range for *N. rhodochorde* juvenile growth is 13-16 ppt, with an optimal average salinity of 13.7 ppt.

This study provides preliminary evidence on the growth response of *N. rhodochorde* juveniles fed fermented nypa palm fronds using cellulolytic bacteria NrLtC4 and NrLtG2. However, it has limitations: the small laboratory-scale experiment may not reflect natural or production conditions; no detailed biochemical feed analysis was performed, limiting understanding of nutritional improvements; and long-term sustainability factors—such as nutrient stability, metabolite safety, and ecological impact—were not assessed yet. Future research should involve larger-scale trials, biochemical validation, and sustainability evaluations to ensure broader applicability of the fermented feed system. Future research will address current limitations by expanding experimental scale, performing biochemical feed analyses, and evaluating the long-term sustainability of the fermented feed system. The next phase involves semi-production trials with larger populations under near-natural conditions to assess the consistency of *N. rhodochorde* growth. Comprehensive nutritional profiling—including amino acid composition, fatty acid profile, and metabolizable energy—will clarify the nutritional benefits conferred by cellulolytic bacteria.

Studies on feed stability, metabolite safety, and environmental impact are essential for assessing long-term viability. Emphasis should be placed on practical applications and scaling up, particularly using fermented nypa palm fronds as a sustainable local feed for nypa palm worm aquaculture in coastal and mangrove systems. This approach can reduce feed costs, promote circular bioresource use, and enhance the sustainability of coastal aquaculture, especially in West Kalimantan.

CONCLUSION

The utilization of Nypa palm fronds fermented by cellulolytic bacteria strains NrLtC4 and NrLtG2 resulted in enhanced growth performance compared to the consumption of unfermented Nypa palm fronds. Specifically, juveniles that were fed nypa palm fronds treated with the fermented isolate NrLtG2 exhibited superior growth metrics, with an absolute segment gain of 121.52 segments and a specific segment gain of 4.63%. Additionally, the body length gain recorded was 30.50 mm, which corresponds to a specific body length growth rate of 74.10 mm/day. While the fermented Nypa palm fronds did not significantly influence survival rates, the highest survival rate among juveniles was noted in those fed Nypa palm fronds with the fermented isolate NrLtC4, achieving a survival rate of 72%. These findings suggest potential for fermented nypa palm biomass in polychaete nutrition, though further validation is needed to account for strain variability, sample size limitations, and environmental influences before broader application in aquaculture systems.

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AUTHOR CONTRIBUTIONS

HPS: collected and analyzed data and wrote the manuscript. AHY: supervised the entire process and contributed to the manuscript. TRS: designed the research and performed data analysis. RK: supervised the manufacturing of the fermentation starter and supervising the manuscript.

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