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Short Communication

Nypa Palm Frond Degradation Utilizing Cellulolytic Lactic Acid Bacteria Isolated from Nypa Palm Worm (*Namalycastis rhodochorde*)

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ABSTRACT
Cellulolytic lactic acid bacteria (LAB) isolated from the gut of nypa palm worms demonstrated cellulase enzyme activity. The primary objective of this study was to investigate the cellulose degradation capabilities of nypa palm frond of three LAB treatments and varying bacterial concentrations (2 %, 3.5 %, and 5 %) over a duration of 30 days. The results indicated that the NrLtG2 isolate exhibited superior degradation capabilities, resulting in a reduction of cellulose content ranging from 20.14 % to 26.39 %. In contrast, the NrLtC2 and NrLtC4 isolates displayed lower degradation abilities, with reductions ranging from 12.50 % to 15.63 %.

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Nypa palm worms (*Namalycastis rhodochorde*) represent a specific category of soil macrofauna that exclusively inhabit mangrove forest ecosystems characterized by the Nypa palm trees (*Nypa fruticans*). The worm is utilized by locals as fishing bait and as a feed source for fish, owing to their significant economic value attributed to their rich amino acid and fatty acid composition. The increasing demand for Nypa palm worms within these communities, coupled with the direct exploitation of this resource, poses a threat to their populations in natural habitats (Junardi 2008; Junardi & Riyandi 2020).

Anticipatory measures aimed at reducing the presence of *N. rhodochorde* in the environment are implemented through ongoing cultivation practices of *N. rhodochorde*. Junardi and Riyandi (2020) conducted experiments on feed that improved the efficacy of *N. rhodochorde* and facilitated the addition of segments using EM4 fermentation of nypa palm fronds. Previous research by Yanti et al. (2020) identified ten distinct types of cellulolytic LAB present in the gastrointestinal tract of *N. rhodochorde*. Among these, three isolates exhibited a high cellulolytic index, encoded NrLtC 2, NrLtC 4, and NrLtG 2. The capacity of these indigenous isolates to produce cellulase enzymes in degrading CMC renders them particularly suitable for application as biodegradation agent of nypa palm fronds for nypa palm worm feed in cultivation.

Microbes that degrade Nypa palm fronds can improve the nutritional content needed for *N. rhodochorde* in cellulolytic LAB cultivation media (Setyawati et al. 2021). These microbes are commonly found in isolation in the gastrointestinal tract of nypa palm worms, providing a potential solution. The lack of research on the cellulolytic LABs ability from the gastrointestinal of Nypa palm worms to degrade Nypa palm fronds underscores the necessity for this study. This research is necessary to assess the effectiveness of isolates in degrading nypa palm fronds. Feeding *N. rhodochorde* with the available nutrients is essential for achieving maximum yields. This research aims to determine the rate of the cellulose degradation activity of cellulolytic bacteria isolated from the digestive tract of Nypa palm worms when exposed to Nypa palm fronds. This research was undertaken to provide foundational support for the development of bacterial isolates as agents for the biodegradation of cellulose fibers and to explore their potential as components of probiotic feed, specifically targeting the biodegradation of Nypa palm frond fibers for the cultivation of nypa palm worms.

Nypa palm leaf fronds were collected from the secondary mangrove area of Sungai Kakap Village, Kubu Raya Regency, West Kalimantan. Three isolates of LAB with cellulolytic activity against CMC were isolated by Yanti et al. (2020) from the nypa palm worm, which was utilized in the degradation process. This study employed an experimental design comprised two treatments: I1 = NrLtC2, I2 = NrLtC4, and I3 = NrLtG2, alongside three concentrations of the isolate: K1 = 2 %, K2 = 3.5 %, and K3 = 5 % with three replications. The three isolates were cultivated in de Man, Rogosa, and Sharpe (MRS) broth media for 24 hours. The treatment starter suspension was prepared using a dilution method, which involved counting the number of colonies with the standard plate count method until the concentration reached 10^7 CFU ml^{-1}.

The dried Nypa palm fronds weighed 100 grams and were placed into a container with 1250 ml of distilled water. The three starter isolates were put into containers with bacterial concentrations of 2 %, 3.5 %, and 5 %, respectively, each with three repetitions. The sample was then incubated at room temperature in an airtight environment for 30 days (Egwuatu & Appeh 2018). During fermentation, bacterial density was observed every week. Following fermentation process, alterations in the cellulose content of palm leaf fronds, bacterial density, and pH levels of the substrates were assessed by comparing measurements taken before and after treatment (Yusnia et al.

2019). The degraded cellulose content was determined using the following formula:

$$\mathbf{KST}\ (\%) = \frac{(\mathbf{KS0}\ (\%) - \mathbf{KSi}\ (\%))}{\mathbf{KS0}\ (\%)}$$

Information: KST = Degraded cellulose content; KS_0 = Initial cellulose content; KSi = Final cellulose content

Determining cellulose content was conducted by modifying the Datta Method (2024). One gram of dry sample was added to 150 ml of distilled water and refluxed at 100 °C using a water bath for an hour. The results are filtered to obtain a residue and then washed with 300 ml of hot water. The residue was then dried in an oven until it reached a constant weight (weight b). Subsequently, the residue was combined with 150 ml of 1 N H_2SO_4 and refluxed in a water bath for 1 hour at a temperature of 100 °C. The results were filtered and washed with 300 ml of neutral solution, and the residue was dried until it reached a constant weight (weight c). The dry residue was added to 10 ml of 72 % H_2SO_4 and soaked at room temperature for 4 hours. The residue was mixed with 150 ml of 1 N H_2SO_4 and refluxed at 100 °C using a water bath for 1 hour. The residue was filtered and washed with 400 ml of distilled water until neutral. The residue is then heated in an oven at 105 °C until the weight is constant and weighed (weight d). Next, the residue is ashed and weighed (weight e) (Chesson 1981). Determination of cellulose content was carried out using the formula:

Cellulose content (%) = (c - d) / a x 100 %.

The analysis result showed that the initial cellulose content in the fiber of nypa palm fronds was 48 %. Following treatment with cellulolytic bacteria over a 30-day incubation period, the cellulose content exhibited a reduction ranging from 12.50 % to 26.39 %. The results of the ANOVA analysis indicated that the treatment effects of various bacterial types and bacterial suspension concentrations did not differ significantly from the control group, which underwent natural degradation without sterilization of the nypa palm fronds. However, an examination of the percentage values for cellulose reduction revealed that treatment I3K1 (isolate NrLtG4 at a concentration of 2 %) achieved the most substantial decrease in cellulose content, amounting to 26.39 %. In contrast, Treatments I1K1 (isolate NrLtC2 at a concentration of 2 %) and I1K2 (isolate NrLtC2 at a concentration of 3.5 %) demonstrated only a modest reduction in cellulose content, with decreases of 12.50 %. Notably, isolate NrLtG2 exhibited the highest level of cellulose reduction among the tested isolates (Table 1).

The bacterial isolates NrLtC2 and NrLtC4, which were isolated from coelomic fluid, and NrLtG2, isolated from the gastrointestinal tract, were selected as starters because previous studies showed that they had a high cellulolytic index (>2) (Yanti et al. 2020; Setyawati et al. 2021). The bacterial isolate NrLtG2 exhibited the highest cellulolytic index value when compared to the isolates NrLtC2 and NrLtC4. The elevated cellulolytic activity observed both in vitro in carboxymethyl cellulose (CMC) media and in the direct degradation of nipah fronds can be attributed to the NrLtG2 isolate, which was sourced from the intestines of worms that were in direct contact with nipah fibers due to their deposit feeding behavior. The bacterial isolate NrLtG2, residing in the digestive tract, plays a crucial role in the processes of food digestion and nutrient absorption, as these processes occur within the digestive system. In contrast, coelomic fluid typically contains nutrients that support the surrounding tissues; consequently, the microbial activity within the coelomic fluid is generally less pronounced in terms of depolymerization activities (Nakagawa et al. 2017). Bacteria can enter the digestive tract

primarily through feeding activities, particularly during the deposit feeding process. However, in species such as nypa palm worm and several other annelids, bacteria may also colonize the coelomic cavity. The presence of bacteria in the coelomic cavity and fluid is believed to result from the movement and transfer of bacteria from the intestines. Research conducted by Yakkou et al. (2021) and Zhong et al. (2022) demonstrated the colonization of bacteria isolated from the coelomic fluid of earthworms and probiotic *Bacillus* from coelomic fluid of peanut worm (*Sipunculus nudus*). However, there is a lack of detailed information regarding the mechanisms by which bacteria infiltrate the coelomic cavity, which serves a critical role in the immune system of the worm. Bacteria in the gastrointestinal tract can ferment food ingredients that are not digested by the body, such as fiber, and produce compounds such as short-chain fatty acids that can be utilized by the body (Holscher 2017; Nogal et al. 2021; Dar et al. 2021).

Table 1. Cellulose content after 30 days of incubation.

Treatments	Initial Cellulose Content (%)	Final Cellulose Content average (%)	Cellulose Reduction average (%)
Control	48,00 ± 0.00	40.33±1.15	15.97±1.41
I_1K_1		42.00±2.00	12.50±2.17*
I_1K_2		42.00±1.00	12.50±2,08*
I_1K_3		40.50±3.06	15.63±3,51
I_2K_1		40.00±1.00	16.67±2.08
I_2K_2		38.83±2.75	19.10±3.00
I_2K_3		36.83±4.37	23.26±5.15
I_3K_1		35.33±2.00	26.39±2,36**
I_3K_2		38.33±0.58	20.14±0.77
I_3K_3		36.33±2.08	24.31±2.43

Footnotes: The treatment consists of a combination of bacterial strains (I1K1 = NrLtC2 with concentration 2 %; I1K2 = NrLtC2 with concentration 3.5 %; I1K3 = NrLtC2 with concentration 5 %; I2K1 = NrLtC4 with concentration 2 %; I2K2 = NrLtC4 with concentration 3.5 %; I2K3 = NrLtC4 with concentration 5 %; I3K1 = NrLtG2 with concentration 2 %; I3K2 = NrLtG2 with concentration 3.5 %; I3K3 = NrLtG2 with concentration 5 %. The asterisk (*) denotes the lowest reduction, while the double asterisk (**) the highest reduction.

The bacterial isolates added during incubation in all treatments experienced increased cell growth until the 4th week. Cell growth occurs exponentially, with the highest increase in the 3rd and 4th weeks. The highest cell growth occurred in the I2K2 (NrLtC4 isolate with concentration 3.5 %) treatment which reached a cell number of 8.7×10^9 CFU ml^{-1} in the 4th week, while the lowest increase was observed in the I3K2 (NrLtG4 isolate with concentration 3.5 %) treatment, with a cell number of only 2.8×10^9 CFU ml^{-1} in the 4th week (Figure 1).

The medium used to test nypa palm frond degradation did not show a significant change in pH value. The pH value remains neutral from before incubation until completion on day 30, ranging between pH 6.00 and 7.00 (Figure 2). Changes in the degree of acidity occur in tiny increments. The data regarding the number of bacterial colonies demonstrate an inverse relationship with the pH value of the substrate. Specifically, an increase in the number of cells corresponds with a decrease in the pH value of the substrate observed during the fourth week (Figures 1 and 2). However, the reduction in the substrate's pH has not been substantial enough to act as an inhibitor of LAB growth. This phenomenon is believed to be influenced by the suboptimal breakdown of cellulose within the degradation system, as well as a relatively low fermentation rate of organic acids (Othman et al. 2017).

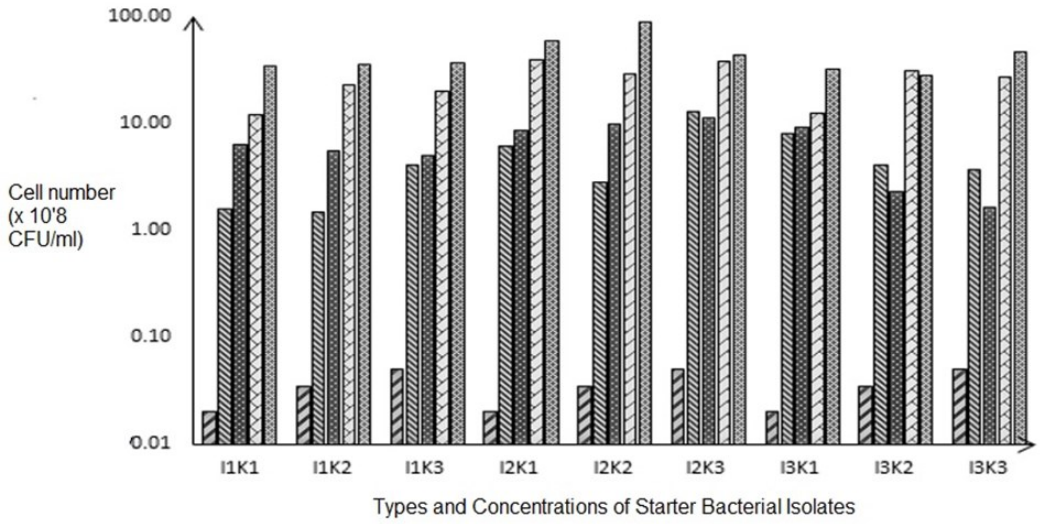

Figure 1. Growth of bacterial isolates during incubation, isolate NrLtC2 (I1), isolate NrLtC4 (I2), isolate NrLtG2 (I3), 2 % (K1), 3.5 %, and (K2), 5 % concentration (K3), in the week 0 , 1st week , 2nd week , 3rd week , 4th week . The treatment consists of a combination of bacterial strains (I1K1 = NrLtC2 with concentration 2 %; I1K2 = NrLtC2 with concentration 3.5 %; I1K3 = isolate NrLtC2 with isolate concentration 5 %; I2K1 = NrLtC4 with concentration 2 %; I2K2 = NrLtC4 with concentration 3.5 %; I2K3 = NrLtC4 with concentration 5 %; I3K1 = NrLtG2 with concentration 2 %; I3K2 = NrLtG2 with concentration 3.5 %; I3K3 = NrLtG2 with concentration 5 %.

Based on the conducted research, it can be concluded that the cellulolytic lactic acid bacteria isolates designated as NrLtC2, NrLtC4, and NrLtG2 possess the capability to degrade nypa palm fronds. It is suspected that these bacteria originate from the interaction between the digestive tract and cellulose substrates, which are consistently present due to the deposit-feeding activity of nypa palm worms (Setyawati et al. 2021). However, the degradation efficiency of these three isolates remains relatively low, with degradation rates ranging from 12.50 % to 26.39 % over a period of 30 days (Table 1). This phenomenon is believed to result from variations in the types of cellulose-degrading enzymes produced by the three bacterial isolates, which have not yet been thoroughly investigated. Additionally, the presence of other complex compounds may directly inhibit the cellulose degradation process. Tamunaidu and Saka (2011) characterized the chemical composition of nypa palm, which includes fronds, shells, husks, and leaves, analyzing components such as cellulose, hemicellulose, lignin, starch, protein, extractives, and inorganic constituents for each part. The total chemical composition revealed that the cellulose and hemicellulose contents ranged from 28.9 % to 45.6 % and 21.8 % to 26.4 %, respectively, with hemicellulose being predominantly composed of glucuronoxylan. The lignin content was found to be between 19.4 % and 33.8 %. In addition to these primary chemical components, starch, protein, and extractives were also present in significant quantities, ranging from 2 % to 8 %. Among the three isolates, NrLtG2 exhibited the highest degradation activity, achieving a cellulose degradation rate of up to 26.39 %, whereas the NrLtC2 isolate demonstrated the lowest degradation activity, with a cellulose degradation rate of less than 20 % (Table 1).

The degradation ability of the bacterial isolate illustrates the potential of indigenous cellulolytic LABs as a biodegradation agent in the production of feed for nypa palm worm cultivation. However, the results obtained in this study were suboptimal, as the degradation percentage remained below 39.57 %, a benchmark established by *Bacillus* DC-11 in the research conducted by Li et al. (2023). Datta (2024) observed that cellulose degradation is a gradual

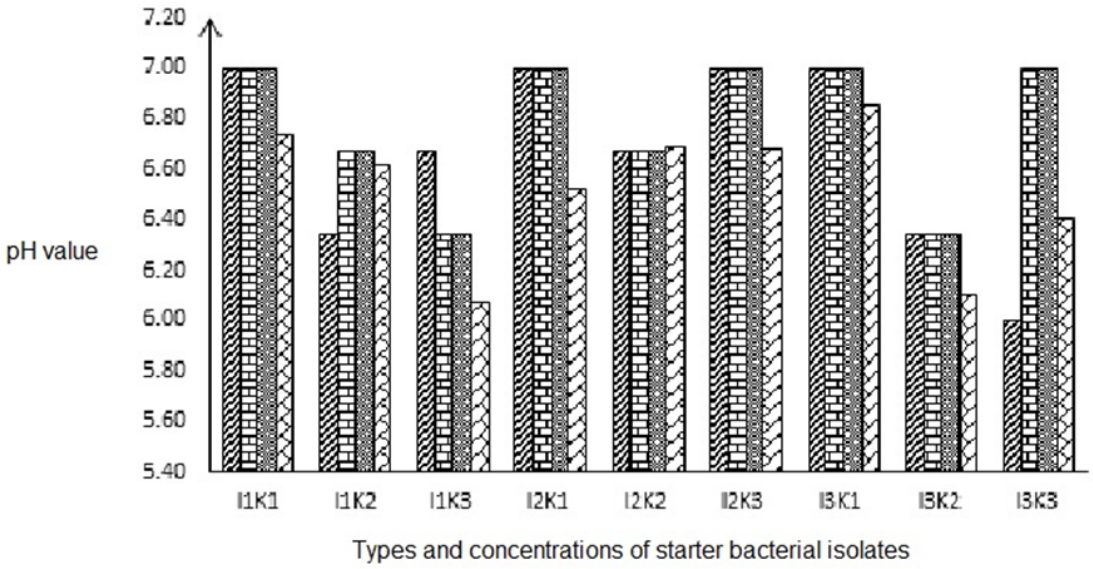

Figure 2. Changes in the pH value of the media during the incubation process in the 1st week , 2nd week , 3rd week , and 4th week . The treatment consists of a combination of bacterial strains (I1K1 = NrLtC2 with concentration 2 %; I1K2 = NrLtC2 with concentration 3.5 %; I1K3 = NrLtC2 with concentration 5 %; I2K1 = NrLtC4 with concentration 2 %; I2K2 = NrLtC4 with concentration 3.5 %; I2K3 = NrLtC4 with concentration 5%; I3K1 = NrLtG2 with concentration 2 %; I3K2 = NrLtG2 with concentration 3.5 %; I3K3 = NrLtG2 with concentration 5 %.

process influenced by a multitude of factors that can affect the degradation process both directly and indirectly. These factors encompass the availability of nitrogen, temperature, aeration, moisture content, pH, lignin content, as well as the concentration and activity of cellulases. Therefore, further research is necessary to determine the optimal concentration of LAB, the appropriate combination of lignin-degrading agents, and other compounds present in nypa palm fronds. Additionally, system is required to enhance the fermentation performance for Nypa palm fronds.

AUTHOR CONTRIBUTION

R.K; T.R.S; A.H.Y; and A.W. designed the research, collected and analyzed the data, supervised all the process, and wrote the manuscript.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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