Enzymatic Activities of *Streptomyces* spp. Isolated from Natural Habitat of Nipah Worm in Sungai Kakap District, West Kalimantan

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Abstract. *Streptomyces* involves in the degradation process of organic compounds on the soils, including mangrove forests. In this process, *Streptomyces* secretes several enzymes to convert the substrates into other substances, which will readily be available for detritus feeders, including nipah worms (*Namalycastis rhodochorde*). This study investigated the enzyme activities of six *Streptomyces* spp. that has been isolated from mangrove soil in Sungai Kakap District, West Kalimantan. Enzymatic activities were detected on four different media: CMC-congo red agar, skim milk agar, gelatine nutritive agar, starch casein agar. Catalase activity was detected using 3% of H₂O₂ solution. The results showed that six *Streptomyces* spp. had proteolytic, cellulolytic, amylolytic, gelatin liquefaction, and catalase activities. *Streptomyces* NrASA1 has the highest proteolytic activity index values, namely 0.57. *Streptomyces* NrASA3 and *Streptomyces* NrASA4 have the highest amylolytic and cellulolytic activity index values, respectively 0.76 and 1.15. The enzymatic activity profile of indigenous *Streptomyces* spp. can be utilized for the development of feed formula for nipah worm cultivation.

1. Introduction

Actinomycetes are soil microorganisms that were spreading in various types of soil. Its growth ability is also broad against several physical and chemical factors, which affect its physiological activity. It is well known that actinomycetes live as a saprophyte and actively decompose organic matter, thereby increasing soil nutrient content. The ability of actinomycetes to degrade organic compounds is due to enzymatic activity. Enzymes as a primary metabolite product can be released into the environment through the process of extranuclear enzymes [1] [2].

The type of Actinomycetes depends on soil structure, physical characteristics, organic substrate, and environmental pH value. The amount of Actinomycetes usually increases in the presence of decomposition of organic material [3]. The mangrove forest is an important area that has high productivity and organic matter content. It is home to mangrove vegetation and many marine animals so that it contains various protein compounds as well as starch and cellulose biomass. Actinomycetes is one type of bacteria that can be found in this region, including *Streptomyces* genera [4]; [5].

*Streptomyces* is a genus that has a wide habitat character and adaptation niche. Although some literature and research suggested that *Streptomyces* were not suitable for growing on wet soils, it was different from the results of our previous research. Previous studies had shown the density of...
Streptomyces spp., which was high in mangrove sediments that having watery soil character [5]; [6]. The previous study showed that 12 isolates of Streptomyces were successfully isolated from nipah mangrove areas in the Kakap River, Kubu Raya Regency, West Kalimantan. Nipah (Nypa fruticans) is one of mangrove vegetation that commonly grows in low salinity habitat [7]. Areal with high organic material productivity, such as nipah mangrove, could increase the chance of finding Streptomyces to be isolated to obtain actinomycetes in the aquaculture field.

Nipah worm cultivation is one of the potential cultivation to be developed in West Kalimantan. However, until now, the cultivation of nipah worms was still constrained by its growth [8]. Worm cultivation that has been conducted on a laboratory scale before showed the nipah worm growth, which was relatively slow [9]. Nipah worm growth depends on organic compounds, especially derived from the decomposition of the palm tree. Palm fronds that decompose in mangrove sediments is a substrate for microorganisms such as Streptomyces, which produces extracellular enzymes activities cellulolytic, amyloytic, gelatin liquefaction, and proteolytic.

Adult nipah worms are a group of benthic organisms with a dietary deposit feeder, which eats organic material deposited in sediments. Many soil-dwelling bacteria are known to produce primary and secondary metabolites to suppress microorganisms that compete for the same resources [10]. Streptomyces is the most distributed microbes that inhabit the soil environment and have a function in degrading organic compounds through enzymatic activities. For this reason, it was needed to investigate enzymatic activities, such as cellulolytic, amyloytic, and proteolytic, catalase activity, and gelatin liquefaction. Streptomyces can be used in the enzymatic process of feed ingredients for producing supplement feed products that contain probiotics and simple nutrients (prebiotics) degraded by microbes. We hope that it could be an effective source of energy to support growth and as a strategy in aquaculture to prevent bacterial infections and could replace antibiotic and chemotherapeutic use.

2. Methods

2.1 Sterilization of equipment and culture medium preparation

The equipment used in the research, such as Petri dishes, measuring glass, test tubes, and borosilicate glass, are washed with detergent then rinsed with water until clean and dried. Media that used for inoculation and test of the Streptomyces were CMC agar (MgSO\(_4\).7H\(_2\)O 0.5 g/l, NaCl 2.3 g/l, Na\(_2\)HPO\(_4\).2H\(_2\)O 5 g/l, Yeast extract 2 g/l, CMC 10 g/l, and Agar 15 g/l), skim milk agar (peptone: 5 g/l, beef extract 3 g/l, skim milk 5 g/l and agar: 15 g/l), starch basal (K\(_2\)HPO\(_4\) 3 g/l, KH\(_2\)PO\(_4\) 3 g/l, MgSO\(_4\) 3 g/l, NaCl 5 g/l, and starch 10 g/l), gelatin nutritive agar (102 g/l) and glycerol asparagines agar (l-asparagine 1 g/l, K\(_2\)HPO\(_4\) 1 g/l, glycerol 10 ml/l, FeSO\(_4\) 0.1 g/l, ZnSO\(_4\) 0.1 g/l, MnSO\(_4\) 0.1 g/l, and agar 17 g/l). The equipment and media were sterilized in an autoclave at 121°C at 0.15 Mpa for 15 minutes.

2.2 Pure cultured of Streptomyces spp. preparation

Six pure isolates of Streptomyces spp. inoculated to asparagine agar glycerol media by a continuous streak method. The pure isolates of Streptomyces spp. were then incubated for three days at a temperature of 30°C.

2.3 Testing the enzymatic ability of Streptomyces spp. isolated from nipah mangrove sediments

Testing the enzymatic ability of Streptomyces spp includes testing of cellulolytic activity, proteolytic activity, amyloytic activity, decomposition of gelatin, and the breakdown of H\(_2\)O\(_2\) [11].

2.3.1 Cellulolytic activity test

The cellulolytic activity test was carried out by the streak method. Six isolates of Streptomyces spp. streaked with a 6 mm diameter round shape on the CMC agar aseptically. The test media was then incubated for seven days at 30°C. After seven days, the surface of the CMC agar was flooded with 2% red congo solution and then allowed to stand for a few minutes. After that, the red congo solution was rinsed with 10% NaCl solution. The cellulolytic activity was characterized by the appearance of clear
zones around the colony. Clear zone diameters and colonies were measured using calipers. The same thing was conducted with the iodine indicator as a comparison. The cellulolytic index was calculated using the formula as follows: Cellulolytic index: Diameter of clear zone - Diameter of bacterial colony / Diameter of a bacterial colony.

2.3.2 Amylolytic activity test
Amylolytic activity test was carried out by the scratch method. Six isolates of *Streptomyces* spp. streaked with a 6 mm diameter round shape on the starch basal agar aseptically. The test media was then incubated for 7 days at 30° C. After seven days, the surface of the basal starch agar was flooded with Fehling solution and then allowed to stand for a few minutes. Amylolytic activity was characterized by the appearance of clear zones around the colony. Clear zone diameters and colonies were measured using calipers. Amylolytic index was calculated using formula as follows: Amylolytic index: Diameter of clear zone - Diameter of bacterial colony / Diameter of bacterial colony.

2.3.3 Proteolytic activity test
The proteolytic activity test was carried out by the scratch method. Six isolates of *Streptomyces* spp. streaked with a 6 mm diameter round shape on the skim milk agar aseptically. The test media was then incubated for seven days at 30° C. Proteolytic activity was characterized by the appearance of clear zones around the colony. Clear zone diameters and colonies were measured using calipers. The proteolytic index was calculated using formula as follows: Proteolytic index: Diameter of clear zone - Diameter of bacterial colony / Diameter of bacterial colony.

2.3.4 Gelatine liquefaction test
Gelatine liquefaction test was conducted using a stabbed method in a test tube containing gelatine agar. After the inoculation of *Streptomyces* spp, all media were incubated for 7 days at 30° C. Gelatine liquefaction activity was characterized by detecting media still being liquid after storing at 4° C.

2.3.5 Catalase test
The catalase test was carried out by applying *Streptomyces* spp. isolates on glass preparations using ose needles. The *Streptomyces* isolate was dripped with two drops of 3% hydrogen peroxide (H₂O₂) solution. The positive catalase test is characterized by the formation of air bubbles [12].

2.4 Data analysis
This research is descriptive research. Data from the research results are processed and analyzed descriptively by creating a table about characters and enzymatic activity index values for cellulolytic, amylolytic, and proteolytic activities.

3. Results
The ability of bacterial isolates isolated from nipah worms to break down macromolecules of carbohydrates, proteins, inorganic compounds was examined by observing the decomposition of cellulose, skim milk, starch, gelatin, and hydrogen peroxide qualitatively and quantitatively. The measurement parameters of cellulolytic, proteolytic, and amylolytic activity through the measurement of clear zones around the colony (Fig. 1), while the decomposition of gelatin and hydrogen peroxide was investigated by changing the consistency of gelatin into liquid and the formation of bubbles in the catalase activity.
Figure 1. Visualization of the relative index values of cellulolytic (left), amylolytic, and proteolytic (right) activity of *Streptomyces* spp. which is isolated from nipah mangrove sediments, through clear zone parameters (A); gelatin liquefaction and catalase activity identified by simultaneous bubbles (B).

Test results on six pure cultures of *Streptomyces* spp. isolated from nipah mangrove sediments showed that all isolates had cellulolytic and amylolytic activities, but only three isolates had cellulolytic, amylolytic, and proteolytic activities, namely *Streptomyces* NrASA1, *Streptomyces* NrASA3, and *Streptomyces* NrASA4 (Tab. 1).

**Table 1.** Clear zone diameters and index values for cellulolytic, amylolytic, and proteolytic activities of *Streptomyces* spp. isolated from nipah mangrove sediment

| Codes   | Cellulolytic   | Proteolytic | Amilolytic |
|---------|----------------|-------------|------------|
|         | Clear zone (mm) | Index value | Clear zone (mm) | Index value | Clear zone (mm) | Index value |
| NrASA1  | 13.95           | 0.88        | 17          | 0.57        | 20.44          | 0.43        |
| NrASA2  | 22.5            | 0.32        | 0           | 0           | 26.26          | 0.54        |
| NrASA3  | 12.46           | 0.45        | 15          | 0.45        | 29.49          | 0.76        |
| NrASA4  | 17.17           | 1.15        | 16.5        | 0.36        | 22.07          | 0.37        |
| NrASA5  | 24.68           | 0.54        | 0           | 0           | 28.66          | 0.38        |
| NrASA6  | 21.44           | 0.3         | 0           | 0           | 19.78          | 0.12        |

The cellulolytic activity was the only enzymatic activity of *Streptomyces* spp. which has a degradation index value that reached index number 1. Although the value of the clear zone diameter of the amylolytic test was greater than the cellulolytic test, the highest amylolytic index number was only 0.76. *Streptomyces* strain, which has the highest cellulolytic index value, was *Streptomyces* NrASA4. This strain was also a strain that has all three enzymatic activities.

In contrast to *Streptomyces* NrASA4, *Streptomyces* NrASA3, which also had three enzymatic activities, had the highest amylolytic index value. Meanwhile, *Streptomyces* NrASA1 was a strain that has a high proteolytic index value. Like the two previous strains, *Streptomyces* NrASA1 was also a strain that has all three enzymatic activity index values.
Table 2. Presence of gelatin and H₂O₂ breakdown by *Streptomyces* spp. strains isolated from nipah mangrove sediment

| Codes   | Enzymatic activities | Gelatin liquifaction | Catalase |
|---------|----------------------|----------------------|----------|
| NrASA1  | pos                  | pos                  |          |
| NrASA2  | pos                  | pos                  |          |
| NrASA3  | pos                  | pos                  |          |
| NrASA4  | pos                  | pos                  |          |
| NrASA5  | pos                  | pos                  |          |
| NrASA6  | pos                  | pos                  |          |

The results of the gelatin thawing test and the breakdown of H₂O₂ 3% obtained all strains of *Streptomyces* spp. able to change gelatin consistency and break down H₂O₂ into O₂ bubbles through catalase activity (Tab. 2). Liquefaction of gelatin media from semi-solid consistency into liquid still occurred even though the media was stored at 4° C. The catalase activity can be seen through producing O₂ bubbles when H₂O₂ liquid was dropped into *Streptomyces* colony (Figure 1b). All strains of *Streptomyces* showed a high intensity of bubbles resulting from catalase activity.

4. Discussion

Enzymatic activities, including cellulolytic, amylolytic, proteolytic, and gelatinase activity, have been widely studied in order to explore the potential of a microorganism so that it can be utilized in industry and aquaculture. Actinomycetes are one of the most potential microorganisms to be developed because they have primary and secondary metabolites, which are potential for industrial interests. Therefore, many researchers have explored the enzymatic and secondary metabolite activity of this group of bacteria.

A number of research workers in the earlier investigation have also reported that actinomycetes from soil and water bodies possessed a high number of enzymatic activities. [13] have reported protease activity in isolated actinomycetes. Gelatinase activity have reported in isolated actinomycetes [14]. Amylase activity in actinomycetes has been reported by [15], [16], [17], [18], [19] and [20]. Cellulase activity in actinomycetes had been reported by [21], [22], [23], [24]. *Streptomyces* is a group of actinomycetes that have great potential as agents for degrading organic compounds. As previously studied, *Streptomyces* isolated from nipah mangrove sediments were known to have catalase and gelatinase activity (Tab. 2). Catalase and gelatinase activities were the main characters of the genus *Streptomyces*. Those activities are major markers that *Streptomyces* has a wide diversity of enzymatic abilities [25].

About 80% of actinomycetes isolated from marine sediments are *Streptomyces* spp. The *Streptomyces* have been tested to have cellulolytic, proteolytic, amylolytic, and chitinolytic activity [6]. The same thing was found in this study. *Streptomyces* isolated from nipah mangrove sediments in Sungai Kakap Village showed cellulolytic, amylolytic, proteolytic, and gelatinase activity (Table 1). The difference in the results of this study was the ability of cellulolytic and proteolytic. It could be seen that 100% of all strains of *Streptomyces* spp. in this study were able to degrade cellulose, while the [6] said that only 40% of cellulolytic activity was detected from the total strain on the similar analysis method. However, contrary to proteolytic activity, this study detected only 50% of the total number of strains, whereas detected 80% of the total strains.

Different types of sediments or growth substrates of *Streptomyces* certainly affect the enzymatic activity of bacterial cells. *Streptomyces* spp. (NrASA1 - NrASA6) isolated from nipah mangrove sediments had higher cellulolytic and amylolytic activity compared to proteolytic. This was caused by the sediment of nipah mangrove, which is rich in organic material from litter plants. Nipah plants were known to be rich in cellulose biomass and starch [26]. It would be different from marine sediment...
content that is rich in protein. However, the detection of 50% of the proteolytic ability of strains of Streptomyces NrASA1, Streptomyces NrASA3, Streptomyces NrASA4 also proves that strains derived from polysaccharide-rich substrates still have their proteolytic abilities.

5. Conclusion
The conclusion of this study is that all strains of Streptomyces spp. have amylolytic, cellulolytic, gelatinase, and catalase activity. However, only three strains have proteolytic abilities, in addition to the previously mentioned abilities, namely Streptomyces NrASA1, Streptomyces NrASA3, and Streptomyces NrASA4. The enzymatic activity index shows that the three strains can be developed as a degrading agent of complex organic compounds in the production of natural nipah worm feed.

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