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Comparison of *Aspergillus niger* and *Rhizopus oryzae* growth on *Nymphaea pubescens* seed culture media

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Abstract

Potato Dextrose Agar (PDA) is a commonly used medium for the growth of *Rhizopus oryzae* and *Aspergillus niger*. The purpose of the study was to determine *Rhizopus oryzae* and *Aspergillus niger* growth in the fermented process of *Nymphaea pubescens* unhulled seed. The hull of *Nymphaea pubescens* seed consists mostly of lignin and cellulose. The fungi that can grow in the *Nymphaea pubescens* seed fermentation process are expected to accelerate the *Nymphaea pubescens* seed dehulling process. This research is an experimental study with the initial stages of conducting a qualitative test of cellulolytic activity of *Rhizopus oryzae* and *Aspergillus niger*. Then inoculate *Rhizopus oryzae* and *Aspergillus niger* on PDA media and cultivate at *Nymphaea pubescens* unhulled seed. Observation of extracellular cellulases for the formation of clear zones around the cellulase-producing colonies in the cellulolytic qualitative test of *Rhizopus oryzae* and *Aspergillus niger*. The macroscopic observations of the growth hyphae of *Rhizopus oryzae* and *Aspergillus niger*. The results showed that *Aspergillus niger* has cellulolytic activity and can grow in the *Nymphaea pubescens* unhulled seed. But *Rhizopus oryzae* did not have cellulolytic activity and cannot grow in the *Nymphaea pubescens* unhulled seed at fermentation process.

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Introduction

Nymphaea pubescens seeds have long been used by the people of wetland areas in Indonesia (Fatimah *et al*., 2022) and several countries (Aliyu *et al*., 2017) as an ingredient in the manufacture of traditional foods. *Nymphaea pubescens* seed has the main content of carbohydrates, proteins (Aliyu *et al*., 2017), and phytochemical components that have antioxidant activity (Aliyu *et al*., 2018). *Nymphaea pubescens* seeds consist of three main parts, namely macrosclereids, osteosclereids, and endosperm. Lignin and cellulose are the main components of the macrosclereids part of the grain.

Fungi have specific enzymatic for metabolism, including breakdown of the materials for organism growth. *Rhizopus oryzae* and *Aspergillus niger* commonly use Potato Dextrose Agar (PDA) media for cultivation growth. Pre-treatment dehulling using enzymes have been used in several grains, including proteases (Sreerama *et al*., 2009), xylanase, pectinase, and cellulase (Dabhi *et al*., 2019; Murumkar *et al*., 2016). The purpose of the study was to determine whether *Rhizopus oryzae* and *Aspergillus niger* could grow in the fermented process of *Nymphaea pubescens* unhulled seeds. The fungi that can grow in the *Nymphaea pubescens* seed fermentation process are expected to accelerate the *Nymphaea pubescens* seed dehulling process.

Materials and methods

Materials

Nymphaea pubescens seeds collected from Hulu Sungai Utara regency, Kalimantan Selatan province was storage 3 years. *Nymphaea pubescens* seed was dried using sunlight until moisture content 10±1%. Two species of fungi are *Rhizopus oryzae* and *Aspergillus niger* isolate was purchased from Gadjah Mada University, Indonesia. The media were using Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB.

Cultivation and growth condition

Rhizopus oryzae and *Aspergillus niger* were maintained on PDA. The plates were incubated at temperature 30°C for five days for fresh culture isolates. The isolates growth to PDB medium at Erlenmeyer flask to get liquid culture. The medium were incubated at temperature 30°C to get growth optimum condition for spore production and then stored at 4°C until use. For fermentation process, 50g of *Nymphaea pubescens* seeds were added with a suspension of *Rhizopus oryzae* and *Aspergillus niger* 10^6 spore/mL respectively in 50 mL PDB media in a 23 x 16 x high 7cm container, mixed well then covered the container with transparent plastic and fermentation for 72 hours.

The cellulolytic activity test

The qualitative cellulolytic activity test of *Rhizopus oryzae* and *Aspergillus niger* following the method was carried out by observing the presence of a clear zone on 0.1% Carboxymethyl cellulose (CMC) selective solid media (Kusumawati *et al*., 2020) and incubating at room temperature for 5 days. Qualitative test on cellulase solid media containing 0.1%cmC (1.5g Agar in 100 ml of distilled water). Selective media was then added with 5 mL of 0.1% congo red by pouring evenly over the surface of the selective media and left for 1 day, after 1 day the color was washed with 1M NaCl and observations were made. The presence of a hydrolysis zone in the form of a clear zone indicates the activity of the cellulase enzyme.

Results and discussion

The cellulolytic activity

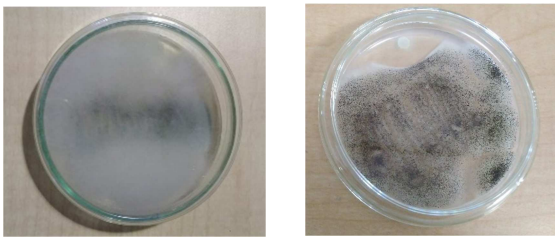
Rhizopus oryzae and *Aspergillus niger* isolates grow on PDA media were able to grow well at room temperature in the range of 30±2°C (Fig. 1). Based on qualitative test on cellulolytic activity in *Rhizopus oryzae* and *Aspergillus niger, Rhizopus oryzae* did not have cellulolytic enzyme activity, while *Aspergillus niger* have cellulolytic enzyme activity (Table 1). Cellulolytic enzyme activity was indicated by the presence of a clear zone on the media containing cellulose (Fig. 2). *Rhizopus oryzae* did not show any cellulolytic enzyme activity, in contrast to *Aspergillus niger* which had cellulolytic enzyme activity used in this study. This is in accordance with research conducted by (Amadioha, 1993; Kusumawati *et al*., 2020) that the activity of cellulolytic enzymes is lower in *Rhizopus oryzae* than in *Aspergillus niger*.

Table 1. Comparative qualitative cellulolytic activity.

Fungi	Cellulolytic activity
Rhizopus oryzae	-
Aspergillus niger	+

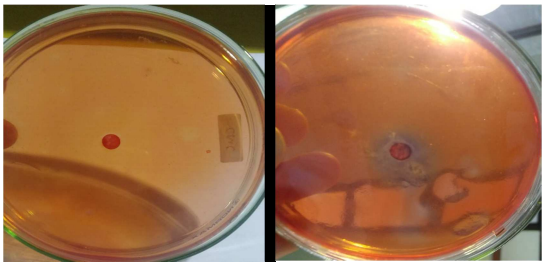
Description : - = did not have cellulolytic activity

+ = have cellulolytic activity



A B

Fig. 1. *Rhizopus oryzae* isolate (A) and *Aspergillus niger* isolate (B) on PDA media.



A B

Fig. 2. *Rhizopus oryzae* (A) and *Aspergillus niger* (B) at the qualitative cellulolytic activity test.

The fungi growth on Nymphaea pubescens seed

Furthermore, *Rhizopus oryzae* and *Aspergillus niger* were used at fermentation on *Nymphaea pubescens* seeds (Fig. 3). The results showed that *Rhizopus oryzae* can not grow well on *Nymphaea pubescens* seed, without fungi hyphae being formed. While *Aspergillus niger* can grow well on *Nymphaea pubescens* seed media with the formation of hyphae in the surface of *Nymphaea pubescens* seed. The inhibition of growth of *Rhizopus oryzae* on *Nymphaea pubescens* seed media because did not have cellulolytic enzymes activity. *Nymphaea pubescens* seed with the main components of lignin and cellulose is not an unsuitable medium for the growth of *Rhizopus oryzae*. This is different with *Aspergillus niger* which can grow well on *Nymphaea pubescens* seed media. This indicates that the cellulolytic enzyme activity of *Aspergillus niger* plays an important role in the ability to grow in fermented *Nymphaea pubescens* seed.

A B C

Fig. 3. *Nymphaea pubescens* seeds (A), *Rhizopus oryzae* cannot grow (B) and *Aspergillus niger* can grow (C) during the fermentation process.

Conclusion

This is the first knowledge to study the use of *Rhizopus oryzae* and *Aspergillus niger* on fermentation at *Nymphaea pubescens* seed. *Rhizopus oryzae* which did not have cellulolytic enzyme activity cannot grow on *Nymphaea pubescens* seed media, while *Aspergillus niger* can grow well on *Nymphaea pubescens* seed media. *Aspergillus niger* have cellulolytic enzyme activity that can grow on *Nymphaea pubescens* seed media.

Acknowledgement

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