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Research Article

Isolation of Indigenous Fungi in River Containing Ammonia from Rubber Industry Waste in Jember

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Abstract
Indigenous fungi are fungi that can degrade organic compounds and make them a source of nutrition for metabolism and life so that they can to survive in various environments including environments polluted by ammonia waste from the rubber factory industry. This study was conducted to obtain fungal isolates that can survive in river water contaminated with ammonia from rubber industry waste in Jember. Isolation was obtained from river water contaminated with rubber factory waste containing ammonia and isolated using the media of Potatoes Dextrose Agar (PDA). The method used in this research is descriptive exploration, namely isolating and culturing fungi using the dilution method. The results of the isolation will identify the genus of fungi based on macroscopic and microscopic morphological characters. Data analysis was done descriptively. The results obtained 4 isolates of indigenous fungi that can degrade ammonia, namely *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and Yeast groups.

Keywords: indigenous fungi, ammonia waste, rubber waste

1. INTRODUCTION

Liquid waste is one type of waste whose presence is often a problem in people's lives [1]. Liquid waste originating from industry generally contains chemical compounds, heavy metals, hazardous and toxic materials (B3), as well as various organic compounds in high concentrations [2].

Indonesia is one of the largest rubber producers in the world, with a total area of 3.6 million hectares (ha) with annual rubber production reaching 3.6 million tons [3]. In addition to having a positive value, if the rubber factory is not processed according to standards, it will produce negative impacts, one of which is as a contributor to ammonia waste in river waters. The high ammonia content is caused by ineffective sewage treatment and still using the anaerobic pond method to treat waste so that only phosphorus, nitrogen, and carbon compounds will be reduced, while ammonia levels are still stored in it [4].

From various wastewater treatment that contains ammonia, biological treatment of ammonia waste can also be carried out. Waste treatment by utilizing microorganisms that can decompose organic substances in the waste. One of the microorganisms that can degrade ammonia waste is the taste of fungal microorganisms. Rubber waste containing ammonia contains various species of indigenous fungi that have the potential to degrade the ammonia compound. These indigenous fungi can be obtained by isolating them from ammonia waste which can later be used as a method in treating ammonia waste in the water. Indigenous microorganisms originating from their substrate habitat have a very high ability to degrade their substrate [5].

Several studies on the isolation of indigenous fungi from water contaminated with the organic matter have been carried out, one of which has succeeded in obtaining 11 isolates of fungi from textile industry waste that have ligninolytic abilities [6]. In addition, other studies were also conducted on the isolation of indigenous fungi from palm oil waste as many as 16 isolates which have the ability as lignocellulolytic agents in the waste [5].

Research on the isolation of indigenous fungi in industrial waste has been carried out, but in addition, research on the isolation of macroscopic and microscopic properties of indigenous fungi in river waters containing ammonia from rubber factory waste in Jember has never been carried out. Based on this background, research on the isolation of indigenous fungi capable of utilizing and degrading ammonia in rubber industry waste needs

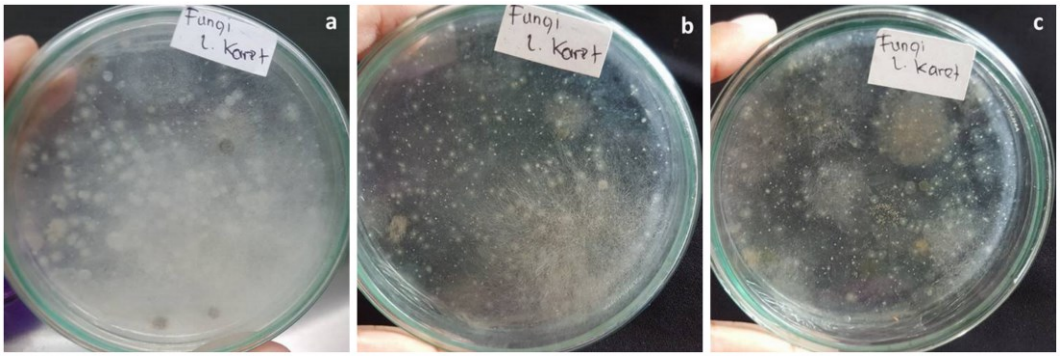

Figure 1. The results of the isolation of indigenous fungi in river water waste contaminated with ammonia. (a) Isolation of fungi at point 1; (b) Isolation of fungi at point 2; (c) Isolation of fungi at point 3.

to be researched. The purpose of this study was to determine the genus of indigenous fungi that can degrade ammonia waste in rivers contaminated with rubber factory waste in Jember.

2. MATERIALS AND METHODS

2.1. Materials

In this study, the tools used were petri dishes, glass beaker, measuring pipette, Bunsen, pH meter, ammonia level measuring instrument, and autoclave. While the materials used in this study were sterile aquadest, *Potatoes Dextrose Agar* (PDA) media, industrial rubber liquid waste, spirit, cotton, aluminum foil, markers, label paper, wood paper [6]. This research was carried out in July 2021, at the Biology Laboratory of PGRI Argopuro Jember University. The sample used came from a river that is close to the waste from the PTPN XII rubber factory, Glantangan Gardens, Jember. Samples were taken at three points that are closest to the location of the rubber industry waste disposal.

2.2. Methods

2.2.1. Sampling

The sample was obtained from the river behind the residents' house, which contains ammonia from the waste processing of the rubber factory, PTPN XII Jember. A sampling of 100 ml at each point (3 sampling points).

2.2.2. Preparation of PDA

As for making 2.8 g/L PDA media: weighing as much as 2.8 Gram, and putting it into a glass beaker. After that, the media was added with 100 mL of distilled water and heated on a heater until homogeneous. The media of PDA that has been made, then put into Erlenmeyer and tightly covered with cotton and coated with aluminum foil. The media was then sterilized using an autoclave at 1 atm, 121 ºC for 20 minutes. Next, each petri dish is filled with 5 mL of media and wait for it to solidify [7].

2.2.3. Sterilization of Tools and Materials

All tools and materials to be used must be washed and dried beforehand. The mouth of the pipette, beaker glass, and Erlenmeyer was first covered with cotton, while the petri dishes were wrapped in newspaper. Then all the tools are wrapped with aluminum foil and wooden paper/ brown paper. The tools that we will use in this study are sterilized by autoclave 1 atm, 121 ºC for 120 minutes [8].

2.2.4. Isolation of Indigenous Fungi

Fungal isolation was carried out by pouring 1 mL of sample into a petri dish containing 5 mL of PDA media, with a dilution of 10^{-4}. After mixing until homogeneous, then rotate the petri dish slowly on the table to form a figure of eight. After the media and water samples solidified completely, the petri dish was then inverted so that the condensed water did not fall onto the lid of the cup. All petri dishes were then wrapped in paper and stored at room temperature for 3-7 days. After the isolation of indigenous fungi was completed, the fungal isolates were purified using media of Potato Dextrose Agar (PDA). Fungal colonies growing in petri dishes were isolated for each different colony [9].

2.2.5. Characterization of Indigenous Fungi

Identification of fungi refers to reference books,

namely *The Identification of Fungi: An Illustrated Introduction With Keys, Glossary, And Guide to Literature Spi Edition*. Macroscopic observations included the shape, texture, and color of fungal colonies growing on PDA media. For microscopic characters, observations were made using a microscope.

2.2.6. Data Analysis

Data analysis was carried out descriptively based on the morphological characteristics and characteristics of the fungus, which was then identified by referring to the book *The Identification of Fungi: An Illustrated Introduction With Keys, Glossary, And Guide to Literature Spi Edition* [10].

3. RESULTS AND DISCUSSIONS

Based on the results of the isolation of fungi at three sample locations grown on media of *Potatoes Dextrose Agar* (PDA) (Figure 1), 6 types of fungal isolates were obtained. Furthermore, from the isolated fungi, pure isolation was carried out on PDA media which was then identified based on their morphological characters.

Based on the identification of the morphological characters, both macroscopic and microscopic, of the isolated fungi (Figure 2, Table 1), it can be seen that the 6 isolates belonged to the Aspergillus, Penicillium, Fusarium, and yeast groups.

The results of the isolation and identification of fungi from water samples containing ammonia showed that the 6 isolates belonged to the Ascomycota Division with different families. *Fusarium sp.* into the family Netriacceae with *Fusarium sp.*, *Aspergillus sp.,* and *Penicillium sp.* into the family Trichomaceae [11]–[14]. These four genera of fungal isolates are known to be able to survive in water containing ammonia and other organic materials that are used as food sources during cell metabolism.

Indigenous fungi are species of fungi that are capable of degrading several organic compounds that will be used as a source of nutrients in the process of cell metabolism [15]. In places polluted by several organic compounds, including water contaminated with paper mill waste containing ammonia, several species of fungi function as bioremediation agents or as bioremediation agents or mycoremediation. Mycoremediation is one method that uses biological agents, namely fungi, to reduce waste from the environment. The presence of fungi in organic compound waste has enzymatic activity that can degrade pollutant waste [16].

In this research, the genus Aspergillus was isolated from river water which became the waste disposal of the rubber industry, at the age of 2 x 24 hours. Based on macroscopic characteristics, colonies were white-brown with a velvety texture and radial stripes (Fig. 3 a.1, Table 1). The results of this isolation are supported by research that reports that the macroscopic characteristics of *Aspergillus fumingatus* grown on media of PDA appear white filamentous colonies that then turn green with white edges and the lower surface of the colonies is yellow to brown [17].

The microscopic character of *Aspergillus sp.* has septate hyphae and hyaline, spherical conidia (Fig. 3 a.2, Table 1). This microscopic observation is supported by other studies which show the presence of conidia a round to semi-spherical shape with smooth walls, conidiophytes having thick walls and having septate hyphae [18].

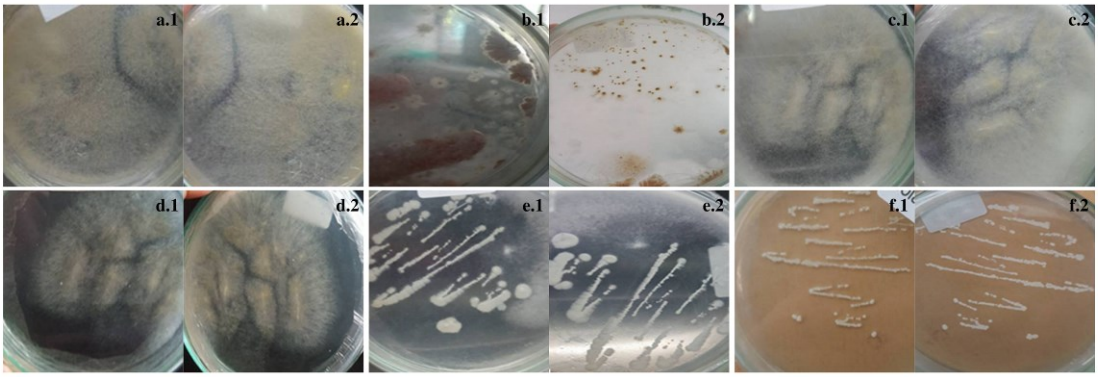

Figure 2. Fungi Isolate at point 1 (a) Isolate 1, (b) Isolate 2; at point 2 (c) Isolate 3, (d) Isolate 4; and at point 3 (e) Isolate 5, (f) Isolate 6; (1 : front view; 2 : back view).

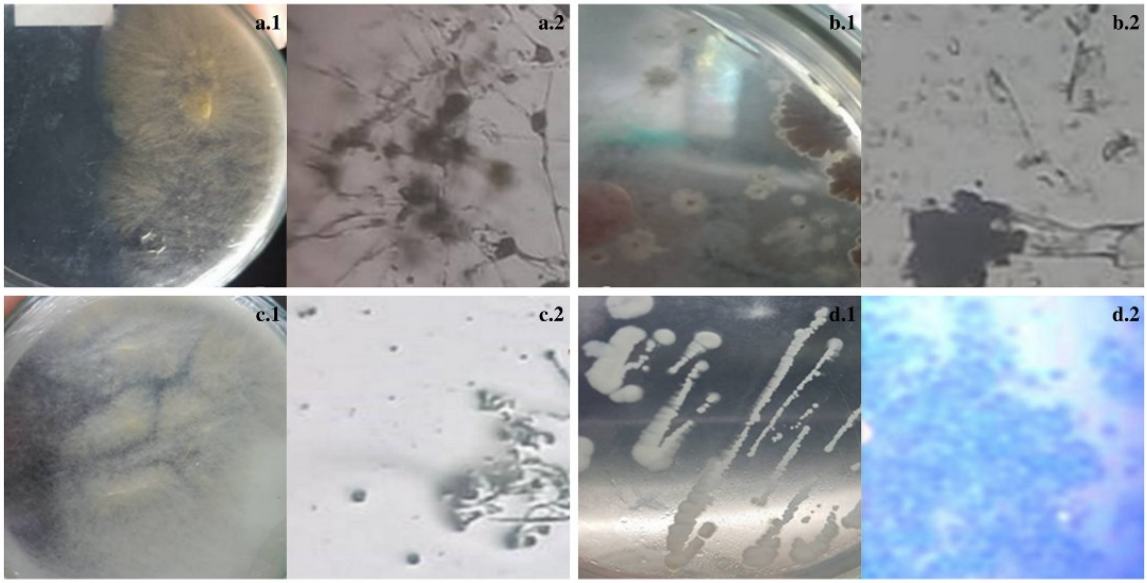

Figure 3. Isolate of Indigenous Fungi that degrades ammonia in 3 x 24 h, a) isolates of *Aspergillus sp.*; b) isolates of *Penicillium sp.*; c) isolates of *Fusarium sp.*; and d) isolate yeast group; (1 : macroscopic, 2 : microscopic).

Aspergillus sp. can dissolve insoluble inorganic compounds by secreting organic acids. *Aspergillus sp.* produces protease enzymes that function in the transformation of organic nitrogen (in the form of protein) in the soil as well as other organic wastes [19]. The ammonia reduction concentration is related to the sulfide reduction concentration in the effluent containing *Aspergillus sp.* under aerobic conditions which will oxidize nitrite to nitrate in a non-toxic form [20]. Fungi of the genus *Aspergillus sp.* is known to be widely used by various studies in degrading waste containing high organic compounds [9].

Isolate *Penicillium sp.* which was isolated for 3 x 24 hours had a Dartmouth green colored colony character with slightly white edges, turquoise cotton -like structure and white edges (Fig. 3 b.1, Table 1). The results of this isolation are supported by research that shows the macroscopic character of the isolate *Penicillium sp.* the cotton-like structure is greenish-white in color and changes color to bottle green with beige edges [17].

In addition, isolates of *Penicillium sp.* the other shows a small green color in the middle and white edges [17]. Microscopic characters of *Penicillium sp.* (Fig. 3 b.2, Table 1), showed that there were smooth-walled conidia, branching conidiophores, metules, stipes, and had septate hyphae. This research is supported by other studies that state that the genus Penicillium sp. has hyaline septate hyphae, rounded conidia, and phialid [21].

Penicillium sp. is a group of fungi that can dissolve phosphate by excreting organic acids produced to release the P bonds from the binding metal [22]. The existence of *Penicillium sp.*, in rivers containing ammonia, cannot be separated from its ability to live and survive in various types of environments. In addition, river water which is used as a rubber factory waste disposal site contains a lot of inorganic and organic substances which make it the best place for the growth of microorganisms including *Penicillium sp.* [23].

Species of *Fusarium sp.*, which had been isolated for 3 x 24 hours showed mycelium colony characters like cotton and brownish-white, not pigmented to the media and showed no radial lines (Fig. 3 c.1, Table 1). This study is supported by observations made by other researchers who isolated *Fusarium sp.* on media of PDA with colonies that have different colors because this fungus is unstable and its DNA is very easy to mutate [24].

In addition, the growth of *Fusarium sp.* on media of PDA and isolated results showed that the macroscopic character of the colonies was brownish white (broken white) [8]. For microscopic characters (Fig. 3 c.2, Table 1) of *Fusarium sp.*, indicating that this fungus had branched or unbranched conidiophores, conidia had many septate and shaped hyphae ovoid. This research was supported by other researchers who observed the fungi *Fusarium sp.* have multiseptal hyphae and

Table 1. Characteristics of indigenous fungi.

Species	Macroscopic		Microscopic				
	Colonies	Texture	Hyphae	Hyaline	Conidia	Metule	Budding
Aspergillus sp	Green with white edges	Velvety	Septate	+	+	-	-
Penicillium sp.	*Dartmouth* green	Cotton	Septate	+	+	+	-
Fusarium sp	*Broken-white*	Cotton	Multi Septate	+	+	-	-
Yeast	Milky-white	Butyrous	-	-	-	-	+

+ : present; - : absence

monophyalid conidiophores, long and unbranched [9][24].

The existence of *Fusarium sp.* on water containing ammonia from industrial processing of the rubber factory showed that this fungus was able to utilize various organic compounds as a source of metabolism. The fungi *Fusarium sp.*, are commonly found in wastewater treatment sediments which are beneficial in increasing the decomposition of organic matter [24]. In addition, this fungus is a heterotrophic microorganism that assists the nitrification process by converting ammonia into nitrate and nitrite, thereby reducing the concentration of ammonia in a bioreactor [25].

The last isolate that was successfully isolated using media of PDA was from the Yeast/Yeast group, which is a polyphyletic group of Basidiomycota and Ascomycota fungi. This fungus has unicellular characteristics and produces buds. Species included in the fungi group are *Saccharomyces cereviceae* and *Candida albicans*. The macroscopic character of this yeast group has a circular shape with convex-shaped elevation, entire margin and milky-white color (Fig. 3 d.1, Table 1). This study was supported by other studies that isolated yeasts with macroscopic characters of circular shape, convex elevation, rough surface, entire margin, milky white and measuring 0.01 - 0.5 m [26]. Microscopic characters are round, elongated ovoid, not pseudohyphae and budding (Fig. 3 d.2, Table 1).

Based on the results of research that has been carried out, it shows that *Saccharomyces cereviceae* which belongs to the yeast group in the anaerobic biofiltration process shows a decrease in BOD, a decrease in total ammonia levels and an increase in COD in polluted water [27]. While for other yeast species, *Candida albicans* can live in the environment. water containing ammonia and has potential as a bioremediation agent [28].

4. CONCLUSIONS

Based on the results of the study, four indigenous fungi isolates were obtained that were able to live in environmental conditions containing ammonia. The four isolates were thought to have the ability to act as bioremediation agents for wastewater containing ammonia from paper mill processing waste, in Jember. Most of the isolates belonged to the Ascomycota and Basidiomycota Divisions, namely *Aspergillus sp.*, *Fusarium sp.*, *Penicillium sp.,* and the yeast group.

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