

## Identification and Characterization of Fungi Associated with Shrimp from Whiteleg Shrimp Ponds in East Java

Fika Zahroh<sup>1</sup>, Shierly Zaissiliya Clarissa Lifani<sup>1</sup>, Rentienna Rachmadhany<sup>1</sup>, Ayu Safira<sup>1</sup>, Rama Nia Julia Putri<sup>1</sup>, Noerita Widya Melati<sup>1</sup>, Yunita Ika Putri Puspitasari<sup>1</sup>, Maria Agustina Pardede<sup>2\*</sup>

<sup>1</sup>Study Program of Aquaculture, Department of Health and Natural Sciences, Faculty of Health, Medicine, and Natural Sciences, Airlangga University, Banyuwangi  
Jl. Wijaya Kusuma No.113, Giri, Banyuwangi, East Java, Indonesia

<sup>2</sup>Sustainability Aquaculture and Environment Research Group, Airlangga University, East Java, Indonesia

**Correspondence:**  
mariapardede@sikia.unair.ac.id

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Fungal infections in shrimp are one of the main challenges in shrimp farming activities that have the potential to reduce productivity levels and crop quality. Fungi are known as opportunistic microorganisms that can cause serious problems, especially when pond environmental conditions are not supportive. Fungal attacks generally occur when shrimp experience physiological stress due to temperature fluctuations, changes in salinity, decreased water quality, or excessively high stocking densities. This study was conducted to identify and characterize the types of fungi associated with shrimp ponds in three main cultivation areas in East Java, namely Banyuwangi, Jember, and Gresik. The fungal isolation process was carried out using Sabouraud Dextrose Agar (SDA) media and incubated at 27°C. Morphological identification was carried out by observing the characteristics of colonies, the shape and color of hyphae, and the structure of conidia. The results of the observations obtained were 6 fungal isolates divided into two main groups, namely yeast including *Candida* sp., *Rhodotorula* sp., and *Brettanomyces* sp. The most commonly found mold species were *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger*. The results of this study demonstrate the potential of fungal probiotics in shrimp farming ecosystems and serve as a basis for developing disease prevention strategies for whiteleg shrimp in East Java's shrimp ponds.

### INTRODUCTION

Indonesia is the largest archipelagic country in the world, with a total water area of 6.4 million km<sup>2</sup>, or two-thirds of Indonesia's total area. Whiteleg shrimp (*Litopenaeus vannamei*) is a leading commodity and an important trade commodity in the world (FAO, 2021; Dugassa & Gaetan, 2018). In 2019, total global whiteleg shrimp production reached 5.44 million tons,

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valued at US\$32.19 billion (FAO, 2021). According to a report by the Food and Agriculture Organization of the United Nations (FAO), total Indonesian whiteleg shrimp production in 2019 reached 677,632 tons and increased to 696,570 tons in 2020. Indonesian shrimp exports reached 251,373.8 tons, valued at US\$2.06 billion. The Ministry of Maritime Affairs and Fisheries is targeting a 250% increase in Indonesian shrimp production, equivalent to 1,290,000 tons, by 2024 (KKP, 2020). According to Farraosi *et al.* (2022), Banyuwangi ponds often employ semi-intensive or intensive cultivation systems. Ponds with these systems often employ high levels of feeding and fertilization, with increasing numbers of DOC (Drowsy Baby Shrimp). This leads to the accumulation of organic matter in the water due to increased feed intake and increased fecal production. High organic matter accumulation can lead to decreased water quality, such as decreased dissolved oxygen levels, increased ammonia levels, and unstable pH changes.

According to Hakim *et al.* (2025), nitrite and ammonia levels exceeding 0.3 ppm cause problems in the cultivation process. These environmental conditions create an ideal habitat for the growth of opportunistic microorganisms, including the fungus *Aspergillus fumigatus*. According to Putra *et al.* (2021), ponds in the Jember region generally have limited water circulation systems, resulting in the accumulation of organic matter from leftover feed and aquaculture waste on the pond bottom. This accumulation causes a decrease in dissolved oxygen and the formation of anaerobic conditions in the sediment. This environment, with its high organic matter content, fluctuating pH, and low oxygen levels, supports fungal growth. Nutrient-rich sediments act as an ideal medium for hyphal growth and fungal spore formation. These conditions not only degrade water quality but also increase the potential for fungal infections in shrimp, especially when the organisms experience stress due to the unstable pond environment. Meanwhile, according to Rohani *et al.* (2016), Gresik ponds are characterized by rice paddy ponds, where the system is traditional. The dry season in the Gresik area causes the pond's salt content to be very low. Low stocking densities allow for reliance on natural food in the form of klekap and plankton. The influence of seasons on water quality fluctuations is closely related. Extreme fluctuations in temperature, pH, and salinity can reduce shrimp's immune system and increase the availability of organic matter from leftover feed and dead organisms, which then serve as a substrate for fungal growth. Thus, seasonal dynamics and water quality instability in traditional ponds in Gresik have the potential to support fungal proliferation in the sediment and water column, which can negatively impact the health of farmed shrimp.

Intensive whiteleg shrimp (*Litopenaeus vannamei*) production can increase pressure on the aquaculture environment and shrimp health. Increased stocking densities, overfeeding, and the accumulation of organic matter and anaerobic sediments create ideal conditions for the growth of opportunistic microorganisms, including fungi. According to Bhassu *et al.* (2024), the shrimp aquaculture sector is highly susceptible to diseases caused by viruses, bacteria, protozoa, and fungi. Environmental factors such as poor water quality, low dissolved oxygen, increased ammonia and nitrite levels, and pH fluctuations can be key triggers for increased fungal disease incidence in shrimp aquaculture. Unstable environmental conditions can accelerate fungal infections because fungi are opportunistic and utilize stressed or injured organisms as entry points (Iqbal *et al.*, 2023). If pond conditions, such as those in Banyuwangi, Jember, and Gresik, have water quality that leads to organic accumulation and limited water circulation, these factors can increase the risk of fungal diseases. This is because the accumulation of organic solids and anaerobic sediment conditions facilitate the proliferation of fungi on the pond bottom and water column, which can then infect shrimp, especially if the

shrimp experience environmental stress (Areshi, 2025). Based on the conditions described, this study aims to evaluate the environmental conditions of shrimp cultivation in the Banyuwangi, Jember, Gresik pond locations that can influence the emergence of fungal diseases, as well as identify the types of fungi associated with farmed shrimp. Therefore, identifying fungi associated with farmed shrimp is important as an initial preventive measure in dealing with fungal disease infections in shrimp.

## METHODS

### Time and Place

This research was conducted in Laboratory 1 of the Faculty of Health, Medicine, and Natural Sciences (FIKKIA) at Airlangga University, Banyuwangi. Sampling was conducted in three vannamei shrimp (*Litopenaeus vannamei*) ponds in different areas: Banyuwangi, Jember, and Gresik. This was due to differences in water sources: Banyuwangi's water source comes from the East Java Sea, Jember's from the Indian Ocean, and Gresik's from the North Java Sea. Sampling was conducted from May to June 2025, with samples taken from traditional and semi-intensive shrimp ponds. The tools and materials used include sample pots, shrimp packing plastic, Bunsen, Laminary Air Flow, micropipettes, microtube racks, microtype racks, petri dishes, vortex, incubator, autoclave, oven, hot plate, spatula, analytical balance, trinocular microscope, paper, measuring cup, aluminum foil, pastel, drigalski, alcohol, styrofoam, ice gel, lighter, section set, tape, Bunsen, beaker glass, Erlenmeyer, vannamei shrimp pond samples, microtubes, microtypes, Phosphate Buffered Saline, Yeast Glucose Chloramphenicol (YGC) media, glass objects, dropper pipettes, SDA media (Sabouraud Dextrose Agar) + Amoxicillin antibiotics, Lactophenol cotton blue, distilled water and oil immersion, and fungal identification books by Robert *et al.* (1981).

### Research Procedures

The study began with sampling from three ponds in Banyuwangi, Jember, and Gresik using sample pots. Next, the equipment and materials were wet sterilized using an autoclave at 121°C for 15 minutes, dry sterilized using an oven at 160°C for 1 hour, and microtubes and microtypes were sterilized using an oven at 80°C for 1.5 hours. The samples were then isolated on Yeast Glucose Chloramphenicol (YGC) media and incubated at 37°C for 48 hours. After fungal colonies had grown, they were separated on Sabouraud Dextrose Agar (SDA) media with the antibiotic Amoxicillin. Macroscopic morphological identification was performed, including color, shape, and texture, and re-incubation at the same temperature and time. After growth, the colonies were further identified macroscopically and microscopically. Macroscopically, three yeast and three mold samples were found. Microscopically, the fungal cells were observed using tape and placed on a glass slide containing one drop of Lactophenol cotton blue. Subsequently, observations were made under a microscope at 400x to 1000x magnification. After obtaining the results, identification was continued using the fungal identification book by Robert *et al.* (1981) and other literature.

## RESULTS

### Fungal Morphology Test

Based on the results obtained, six fungal isolates were found from the whiteleg shrimp ponds, as shown in Table 1.

Table 1. Morphology of Yeast and Mold Fungi from Whiteleg Shrimp Ponds

No.	Name	Colony Color	Microscopic Form
1.	<i>Rhodotorula</i> (1a)	Reddish orange/pink	Elongated oval
2.	<i>Brettanomyces</i> (1b)	Orange-yellow	Elongated oval
3.	<i>Candida</i> (1c)	White/cream	Oval-rounded
4.	<i>Aspergillus flavus</i> (2a), (2b)	White and yellowish green	Conidiophores are clearly visible, unpigmented, rough, less than 1 mm long
5.	<i>Aspergillus fumigatus</i> (3a), (3b)	Dark green, white	Conidiophore stalks are short, and the vesicles are oval and round, becoming oval as the colony ages
6.	<i>Aspergillus niger</i> (3c)	Grayish green, white	Septate hyphae, and asexual spores are conidiospores

### Fungal Morphology Pictures

Based on the morphology of the yeast and mold fungi above, the microscopic results can be seen in Figure 1.

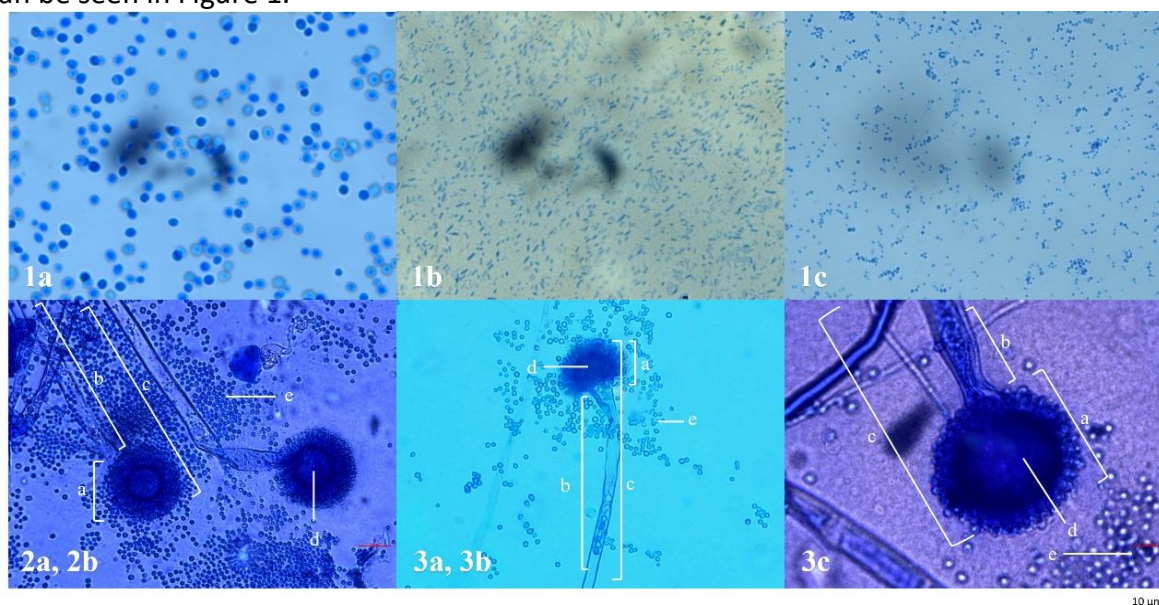


Figure 1. Microscopic Morphology of Yeast and Mold Fungi from Whiteleg Shrimp Ponds. 1a (*Rhodotorula*); 1b (*Brettanomyces*), 1c (*Candida*); 2a, 2b (*Aspergillus flavus*); 3a, 3b (*Aspergillus fumigatus*); 3c (*Aspergillus niger*). Description: a) Conidial Heads, b) Hyphae, c) Conidiophores, d) Vesicles, e) Conidia

The microscopic shape of *Rhodotorula* cells is oval to spherical-elongated, with sizes ranging from  $2.3\text{-}5.0 \times 4.0\text{-}10 \mu\text{m}$ , the cells germinate (budding), commas in the form of short filaments or pseudohyphae (depending on the species and conditions) seen in Figure 1. code 1a (Ochoa-vinals *et al.*, 2022). *Brettanomyces* cells microscopically can be oval to elongated (sausage-shaped or oblong) and sometimes form buds multilaterally (more than one point) and can also form pseudomycelium/pseudohyphae in response to stress conditions with a size of  $5\text{-}7 \mu\text{m}$  seen in figure 1 code 1b (Harrouard *et al.*, 2023). *Candida* sp. has a thin-walled, elongated oval shape, buds, and measures  $2\text{-}3 \times 4\text{-}6 \mu\text{m}$  with budding cell growth and consists of pseudomycelium seen in figure 1. code 1c (Atshan & Shabaa, 2023). *Aspergillus flavus* has hyaline conidiophores and conidia, and can reach a length of 1 mm, has round to semi-round

vesicles and phialids formed directly on the vesicles. The conidia are round or semi-round, pale green and spiny as seen in figure 1 codes 2a and 2b (Zahara *et al.*, 2021). *Aspergillus fumigatus* has a non-septate hyphae morphology, a single conidiophore, and an oval vesicle tip as seen in figure 1. codes 3a and 3b (Asdinar *et al.*, 2024). Meanwhile, *Aspergillus niger* is characterized by brown conidia, black conidia heads, round in shape, and tend to split into columns in old colonies, soft, long, clear conidiophores, grow upright and do not branch, have septate and hyaline hyphae, round to semi-round vesicles and grow directly above the conidiophores, and have sterigmata around the vesicles as seen in figure 1 code 3c (Septiana *et al.*, 2023).

## DISCUSSION

Fungi are a group of organisms that play a vital role in Earth's life systems and ecosystems. Fungi play a crucial role in decomposing organic matter, which is then used as nutrients for their bodies. Fungi lack chlorophyll, are heterotrophic, decomposer, mutualistic, and can also be harmful or parasitic (Solle *et al.*, 2017). Salvamani & Nawawi (2014) suggest that fungi are categorized into two main groups: microscopic fungi and macroscopic fungi. Microscopic fungi are a group of fungi that can only be observed under microscopic magnification due to their extremely small size and cellular structure, while macroscopic fungi have bodies with more complex morphology, allowing them to be observed visually without the aid of optical instruments. Fungi are classified in the Fungi kingdom and are eukaryotic organisms with a membrane-bound nucleus. Fungi are often found in soils with abundant organic matter reserves, as they act as decomposers by decomposing organic matter to obtain nutrients (Mohammad *et al.*, 2024).

Macroscopic fungi are saprophytes that grow in damp areas and low light intensity with substrates such as wood, rocks, soil and decaying leaves (Zunaida & Sulistiyowati, 2022). Macroscopic fungi can be poisonous and non-poisonous (edible). The characteristics of toxic macroscopic fungi (non-edible) are having a prominent cap or top, not being swarmed by ants or other animals, and having a foul odor due to sulfide content (Taribuka & Patty, 2023). Meanwhile, edible fungi can be identified by their flat caps, and are eaten or swarmed by ants (Putri *et al.*, 2024). Microscopic fungi are generally molds and yeasts, where molds are multicellular, filamentous and have mycelium (Larasati *et al.*, 2021). According to Riyanto *et al.* (2024) the characteristics of molds microscopically can be distinguished by the shape of the spores, the type of spores and the type of hyphae. The hyphae of molds are divided into septate (with partitions) and aseptate (without partitions). Yeast, on the other hand, is a unicellular eukaryotic organism belonging to the kingdom Eumycota (Widyaningtiyas *et al.*, 2022). According to Hartati *et al.* (2024), yeast has characteristics that can be seen through the shape, size of the cells, the color of the edges, and the elevation of the colonies. Yeast can be found in various habitats because it has a wide physiological range, allowing it to grow in a wide range of environments (Hartati *et al.*, 2021).

Based on the results obtained, it is known that *Rhodotorula* fungal colonies when grown on agar media often have a salmon-red to reddish-orange color. This is due to the carotenoid pigments produced by its cells (Ochoa-vinals *et al.*, 2022). *Rhodotorula* sp. is a basidiomycota yeast commonly found in aquatic environments. Several studies have shown that *Rhodotorula* has high environmental tolerance (Cespedes *et al.*, 2022). *Rhodotorula* sp. is found widely in environments such as freshwater, seawater, and wastewater. Based on research by Sriphuttha *et al.* (2023), it shows that *Rhodotorula* can also be found on shrimp in tropical waters. This is

due to the conditions of the shrimp cultivation environment which is rich in organic matter, varying salinity conditions, the presence of other microflora, and substrates that allow yeast growth. *Rhodotorula* has not been widely reported as a pathogen for shrimp. Several studies have shown that this genus of fungi has potential as a supplement or probiotic in shrimp and other crustacean cultivation, increasing growth rates and digestive enzyme activity (Zhang *et al.*, 2025). Furthermore, a species of *Rhodotorula* has been used in whiteleg shrimp feed and has been shown to improve growth rates, immune gene expression, gut microbiota, and resistance to *Vibrio parahaemolyticus* infection (Sriphuttha *et al.*, 2023). Poor culture environments (high ammonia concentrations, low dissolved oxygen, and high organic matter content) can trigger the proliferation of opportunistic microorganisms or stress conditions in shrimp, facilitating pathogen attacks (Ochoa-vinals *et al.*, 2022). Although *Rhodotorula* has not been confirmed as a major shrimp pathogen, its presence as a component of the microflora or an indicator of environmental conditions could be relevant. If these pigment yeasts overgrow or biofilms form that disrupt the filtration system, it could indirectly affect water quality or shrimp health (Cespedes *et al.*, 2022). Shrimp cultivation conditions in East Java, including Banyuwangi, Jember, and Gresik, often utilize brackish or seawater ponds, with varying salinity, the presence of organic feed residues, waste residues, and biofilms on pond walls. These conditions can provide a suitable habitat for saprophytic microorganisms such as *Rhodotorula*. *Rhodotorula* sp. has a high tolerance to environmental variations and can grow in both seawater and organic matter. Therefore, in shrimp ponds in Banyuwangi, Jember, and Gresik, its presence is quite likely as part of the environmental microflora.

*Brettanomyces* is a genus of yeast from the Saccharomycetaceae family. Colonies on agar media have general characteristics of yeast, but with a slightly different cell morphology than typical yeast. One distinguishing characteristic of *Brettanomyces* is the flexibility of its cell shape and its ability to adapt to environmental conditions (Williamson *et al.*, 2023). In general, *Brettanomyces* is most often mentioned in the context of the fermentation industry, particularly in the production of wine, beer, and cider, where it is often considered a contaminant/spoilage organism. However, if *Brettanomyces* is isolated from its natural habitat or environment, it likely originates from substrates such as fruit peels, vegetation exudates, bark, and environments with decomposed organic matter (Louw *et al.*, 2016). Suitable habitats for *Brettanomyces* are environments with limited oxygen conditions and organic substrates that allow yeast growth. This allows *Brettanomyces* to live in cultivation environments with freshwater or brackish water cultivation conditions with high organic matter, it is possible that yeast microflora such as *Brettanomyces* are present although not widely reported in shrimp cultivation. Based on Lamçe & Sini (2013), which stated that *Brettanomyces* has not been widely reported to be present in shrimp cultivation ponds or cause serious diseases in shrimp.

*Candida* sp. in SDA media has a characteristic yeast colony form that protrudes from the surface of the media, the texture of the colony surface is smooth and slippery. *Candida* sp. colonies are yellowish white, accompanied by a yeast odor (Nuryati & Huwaina, 2016). *Candida* sp. can be found in mangrove sediments, among others. According to Millatia *et al.* (2022), mangrove sediments are a center of activity for microorganisms such as fungi to obtain nutrients and grow. *Candida* sp. will grow in sediment conditions rich in organic matter, with too low oxygen levels. According to Ochoa-Alvarez *et al.* (2021), *Candida* sp. is a source of beta-glucan in shrimp farming ponds that influences higher mortality rates when shrimp are challenged with WSSV. High organic matter can trigger the growth of *Candida* sp. fungi. This is related to the condition of extensive ponds in Banyuwangi which have a lower N content

than intensive ponds in Banyuwangi (Rasidi *et al.*, 2013). Similar to Banyuwangi, Jember's ponds have a high accumulation of aquaculture waste, leading to anaerobic conditions in the culture. This can trigger the growth of *Candida* sp. in the culture waters, which can be harmful to the shrimp (Putra *et al.*, 2021). Meanwhile, in Gresik, with its traditional ponds, the ponds are adjacent to industrial zones and the calcareous soil substrate causes instability in cultivation activities. Fluctuating pH levels impact shrimp stress levels. This makes *Candida* sp. infections more likely to occur in pond sediments and pond water (Safitri *et al.*, 2020).

*Aspergillus flavus* is a type of fungus that frequently contaminates food. This type of fungus can cause infections in humans and is the fungus that produces the most aflatoxin, a carcinogenic toxin. The macroscopic morphology of *Aspergillus flavus* fungal colonies, according to Gautama (2012), is yellowish-green with white edges, a cotton-like surface, and a yellowish to brown underside. According to Koneman (1992), *Aspergillus flavus* fungal colonies on Potato Dextrose Agar (PDA) media appear to have long and rough conidiophores, round vesicles, and conidia are rarely found. *Aspergillus flavus* is a fungus that produces aflatoxin. The distribution of *Aspergillus flavus* is very wide, generally as a saprophyte in soil and organic material, and can also be found as a pathogen in humans and animals. This fungus grows quickly in a substrate, initially white like cotton and after 2-3 days changes color to yellow or blue/yellowish green. This fungus is commonly found in the Banyuwangi, Gresik, and Jember areas. In the Banyuwangi, Jember, and Gresik areas, it is often found in endoparasites and ectoparasites (Sinaga *et al.*, 2020).

*Aspergillus fumigatus* has a light green, rough-textured colony morphology with thick colonies. *Aspergillus fumigatus* can be found in air, soil, and humus (Asdinar *et al.*, 2024). According to Natasha *et al.* (2022), spores of *Aspergillus fumigatus* can be easily spread through the air, this can cause contamination of feed or grow rapidly in pond sediments and shrimp growth media. High organic matter content in the cultivation media is known to increase the growth of *Aspergillus fumigatus* fungi. High organic matter can be a source of carbon and energy to support fungal metabolism. This condition is related to the characteristics of extensive ponds in Banyuwangi, which have a lower nitrogen (N) content than intensive ponds (Rasidi *et al.*, 2013). Similar to the Banyuwangi region, ponds in Jember Regency also show high accumulation of cultivation waste, which contributes to the formation of anaerobic conditions on the pond bottom. This low-dissolved-oxygen environment can support the proliferation of *A. fumigatus* in aquaculture waters and potentially cause health problems for aquatic organisms, particularly shrimp (Putra *et al.*, 2021). Meanwhile, traditional ponds in the Gresik region are characterized by land adjacent to industrial zones and calcareous soil substrates, which cause fluctuations in water pH and instability in the pond's environmental quality. These pH fluctuations can increase physiological stress levels in shrimp, thereby reducing their resistance to infection by the fungus *A. fumigatus*, which commonly grows in sediment and pond water (Safitri *et al.*, 2020).

*Aspergillus niger* is characterized by its black color, initially white, and spherical colonies. The colonies become darker as they age. The underlayer of *Aspergillus niger* is white or yellow with a thick layer of dark brown to black conidiospores. The surface of the colonies on PDA media in petri dishes is brownish-yellow and consists of several thin fibers, with spherical or semi-spherical colonies. *Aspergillus* can live and be found in various substrates, thus being referred to as a cosmopolitan mold (Mahardhika *et al.*, 2022). Organic matter derived from leftover feed, metabolic waste, dead organisms, fertilizers, liming, and pesticides contribute to water quality degradation. Monitoring results conducted by Primavera (Suwoyo *et al.*, 2015) on shrimp rejuvenation ponds indicate that 15% of the feed provided dissolves in water,

while 85% of the metabolic products are returned to the environment as waste. Organic matter suspended in the water causes turbidity and reduces light penetration, thus affecting oxygen regeneration. Poor water quality can trigger the growth of pathogenic organisms (Sinaga *et al.*, 2020). These fungi are commonly found in ponds in Banyuwangi because changes in weather and climate, such as those experienced in Banyuwangi, can affect pond conditions and increase the risk of production failure, which can be related to an increase in pathogenic microbes such as fungi. The Gresik area has a gentle topography and is close to the sea. The air and water conditions that enter the ponds also easily carry these fungal spores, further facilitating the spread of *Aspergillus* in the shrimp ponds in the area (Sinaga *et al.*, 2020).

## CONCLUSION

Based on the research results, it was found that fungi originating from vannamei shrimp ponds in East Java, especially Banyuwangi, Jember, and Gresik were divided into two main groups, namely yeast including *Candida* sp., *Rhodotorula* sp., and *Brettanomyces* sp. and the types of molds that were often found came from the species *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger*.

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