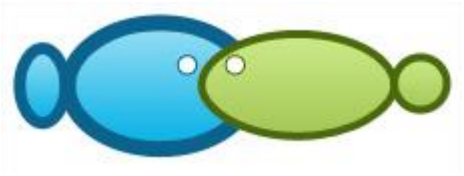

Identification and protease activity of opportunistic pathogenic fungi in giant gourami (*Osphronemus goramy*)

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Abstract. Giant gourami (*Osphronemus goramy*) is a freshwater fish originating from Indonesia and spread in Southeast Asian countries, such as Malaysia. *O. goramy* is a broadly cultivated species that is sold and consumed in Malaysia, with a high economic value. Efforts to increase *O. goramy* cultivation have led to the emergence of various pathogenic microorganisms, such as fungi. Fungal infections occur when *O. goramy* are under certain conditions, so that the fungus is an opportunistic pathogen. The protease enzyme activity test was carried out to determine the proteolytic activity of opportunistic fungal pathogens isolated in *O. goramy*. The study aimed to determine the species and proteolytic activity of opportunistic pathogenic fungal targets in *O. goramy* from the Kampung Gong Badak Fish Market, Kuala Terengganu, Malaysia. The method used in the study was a survey through direct sample collection at the location, carried out by a purposive sampling method. The sample was a healthy *O. goramy*. Fungal identification was performed by macroscopic (visually) and microscopic methods. The protease enzyme activity test was carried out qualitatively by inoculating the fungus on PDA and skim milk media using a sterile toothpick. The results showed that there were seven species of opportunistic pathogenic fungi that were isolated from the gill and kidneys of *O. goramy*. Based on the seven species obtained, three species remained unidentified and four other species were identified as *Exophiala* sp., *Acremonium* sp., *Saccharomyces* sp. and *Penicillium kewense*. Two types of fungus, *Acremonium* sp. and *P. kewense*, showed protease enzyme activity, with a protein hydrolysis index of 1.44 and 1.50, respectively.
Key Words: fungal infections, macroscopic, microscopic, protein hydrolysis index.

Introduction. Giant gourami (*Osphronemus goramy* Lac.) is a freshwater fish originating from Indonesia (Budiana & Rahardja 2018) and spread in Southeast Asian countries, such as Malaysia (Nugroho 2012). According to the data, *O. goramy* is a species of fish that is sold and consumed in Malaysia (Du & Starr 2010) with a fairly extensive rearing cultivation, such as in all districts in Sabah (Inger & Chin 1962). *O. goramy* have a total production volume of 46,877.62 tons, while the overall freshwater commodities reach 163,757 tons, worth about 324 million USD in 2012 (Yusoff 2015). Compared to Malaysia, the production of *O. goramy* in 2012 in Indonesia was 84,681 tons, with an annual production in the subsequent years (until 2016) reaching 149,553 tons (Hardaningsih 2018). Efforts to increase the *O. goramy* cultivation have led to the emergence of various pathogenic microorganisms, such as fungi (Kusdarwati et al 2016). Fungi, as pathogenic microorganisms, can attack and infect *O. goramy* (Khairyah et al 2012).

O. goramy, as a freshwater fish species, can be attacked by *Exophiala* sp. (Gaskins & Cheung 1986), *Acremonium* sp. (Shahraki et al 2014), *Saccharomyces* sp. (Andlid et al 1999), and *Penicillium* sp. (Ito & Abu 1985). However, recent studies reported that other fungal species that often attack *O. goramy* were *Saprolegnia* sp., *Aphanomyces* sp., *Aspergillus* sp., *Alternaria* sp., *Rhizopus oryzae* and *Curvularia lunata* (Khairyah et al 2012). Fungal infections occur when *O. goramy* are under certain conditions, so that the fungus is an opportunistic pathogen, depending on the body resistance, age, disease and previous infections experienced by *O. goramy* (Brown et al

2012). Another factor is the poor water quality. The water quality factor is related to the place where *O. goramy* is found. The studied *O. goramy* specimens were obtained from the Kampung Gong Badak Fish Market, Kuala Terengganu, Malaysia. Ishak (2010) reported that the water quality such as temperature, dissolved oxygen (DO) and the degree of acidity (pH), that differ between the fish market and the cultivation, can affect the presence of fungal infections in *O. goramy*.

However, all previous studies were focused on the identification of fungi in *O. goramy*, in general. To our knowledge, very few studies concerning the identification of opportunistic pathogenic fungi and and their protease enzymatic activity have been conducted. The protease enzymatic activity test was carried out to determine the proteolytic activity of opportunistic fungal pathogens isolated in *O. goramy*. The production of protease enzymes by the fungi can influence the hosts infection process. Based on the background that has been mentioned, it is necessary to know the type and proteolytic activity of the opportunistic pathogenic fungi in *O. goramy* from Kampung Gong Badak Fish Market, Kuala Terengganu, Malaysia.

Material and Method

Isolation of fungi. The isolation technique of opportunistic pathogenic fungi in *O. goramy* was carried out in accordance with the study of Pinzari et al (2010): (1) tools and materials preparation by using a cotton swab; (2) performing the isolation process (in a sterile position near a bunsen flame), by touching a sterile cotton bud to the target organs (gill, kidney, liver, eye, muscle, and liver); (3) performing a swabbing process on the surface of Potato Dextrose Agar (PDA), Mycosel Agar (MA) and Oatmeal Agar (OA) media; (4) wrapping the petri dish with parafilm, then (5) storing the isolated results in an incubator, at 24-25ºC, and (6) waiting for the next 3 days (fungi usually grow for 3-5 days after incubation).

Purification of fungi. The purification process of opportunistic pathogenic fungi in *O. goramy* is first carried out by observing the results of the isolation (visually). If the fungus has grown, then the purification process can be carried out on PDA + antibiotic media that has been made and on Potato Dextrose Broth (PDB) media. The purification process is carried out in a sterile position (near a bunsen flame)as follows: (1) by taking a small amount of fungus from the original medium, using a sterile toothpick that has been autoclaved, then (2) by touching it to the center of the PDA + antibiotic media and PDB media, (3) by wrapping the petri dish with parafilm, (4) by storing the purified fungi in an incubator at 24-25ºC and (5) by waiting until the next day, then (6) by observing the fungus purification results. The fungi purification method using a sterile toothpick was in accordance with the study of Nevalainen et al (2014).

Identification of fungi. The identification of opportunistic pathogenic fungi in *O. goramy* was carried out macroscopically and microscopically (Widiastutik & Alami 2014; Hastuti et al 2015). The macroscopic identification process was carried out by opening the parafilm that encloses the petri dish, then by opening the lid of the petri dish (inside the fume hood), by taking pictures of the fungus and by observing the fungus visually. Then, the microscopic identification process was carried out by dripping lactophenol cotton blue dye (sufficiently) onto the object glass using a micropipette, by taking the mushrooms from the media using a toothpick, by placing the mushrooms on the object glass containing lactophenol cotton blue, by flattening the fungus with lactophenol cotton blue dye (with toothpick) and by covering it with a cover glass. Finally, the fungus was microscopically observed under a microscope with 400x and 1,000x magnification (with immersion oil), and pictures of the fungus visible under the microscope were taken.

Protease activity test. The protease activity test was carried out qualitatively according to the Standard Operating Procedure (SOP) at the Laboratory of Health Aquatic Organisms Universiti Malaysia Terengganu, using PDA media + skim milk as a substrate. The fungal isolates were then inoculated in the center of the media using a sterile

toothpick. Then, the media that had been inoculated with fungi was stored in an incubator at a temperature of 24-25ºC for several days, until a clear zone was formed around the fungal colonies. The proteolytic activity is usually indicated by the presence of a clear zone around the fungal colony (positive test). The clear zone produced in the positive test was the result of the media protein substrate hydrolysis by the protease enzyme produced by the fungal isolates (Yuniati et al 2015).

Results. Opportunistic pathogenic fungi in *O. goramy* from the Kampung Gong Badak Fish Market, Kuala Terengganu, Malaysia were isolated from muscles, kidney, liver, eyes, gill organs and water sample. The fungus was isolated in PDA, MA and OA media, using samples from the kidney and gill of *O. goramy*. No opportunistic pathogenic fungi were found in the muscle, liver, eye and water samples.

The isolates of opportunistic pathogenic fungi were purified on PDA and PDB media. Based on Table 1, there were 7 isolates of purified fungi. The purified fungal isolates were derived from PDA (4 isolates), mycosel agar (1 isolate) and oatmeal agar (2 isolates), as media of origin.

Table 1

Purification results of opportunistic pathogenic fungi in *Osphronemus goramy*

No	*Media of origin*	*Target organ*	*Isolate code*
1	Potato Dextrose Agar (PDA)	Kidney	HKPDA
		Gill	HGI5
		Gill	HGI6
		Gill	HGIIPDA
2	Mycosel Agar (MA)	Gill	HGIII
3	Oatmeal Agar (OA)	Kidney	HKOA
		Gill	HGIIOA

Identification of opportunistic pathogenic fungi. Based on the identification of opportunistic pathogenic fungi in *O. goramy* from Kampung Gong Badak Fish Market, Kuala Terengganu, Malaysia, it was found that the *O. goramy* sample was overgrown by four types of fungi. The first fungus identified as *Exophiala* sp. (McGinnis 1980). This fungus can be purified in PDA media. *Exophiala* sp. has a gray-black color with a smooth velvet-like structure, while the microscopic characteristics are the round conidia and the erect conidiophores (Figure 1).

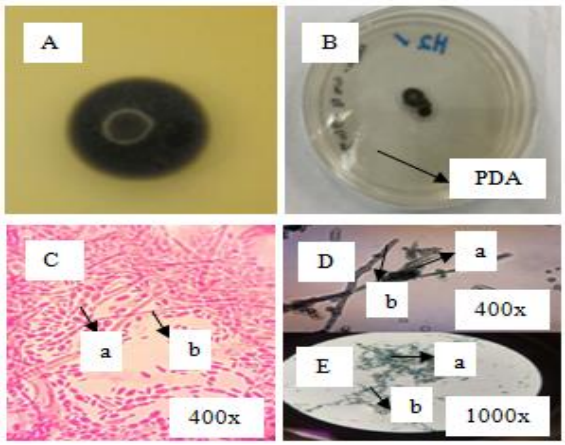

Figure 1. Macroscopic and microscopic characteristics of *Exophiala* sp. (a: conidia, b: conidiophore).

The second fungus identified as *Acremonium* sp. (Domsch et al 2007; Akmalasari et al 2013). This fungus can be purified in PDA media. *Acremonium* sp. has pale, orange colonies, a dense texture (in the middle) and wavy edges, while the microscopic

characteristics are a spherical conidia, an elongated phialid, and erect conidiophores (Figure 2).

The third fungus identified as *Saccharomyces* sp. (Kurtzman & Fell 1998; Widiastutik & Alami 2014). This fungus can be purified in PDA media. *Saccharomyces* sp. has white colonies, a round shape, and a soft texture, while the microscopic characteristics are the spreading spherical spores (Figure 3).

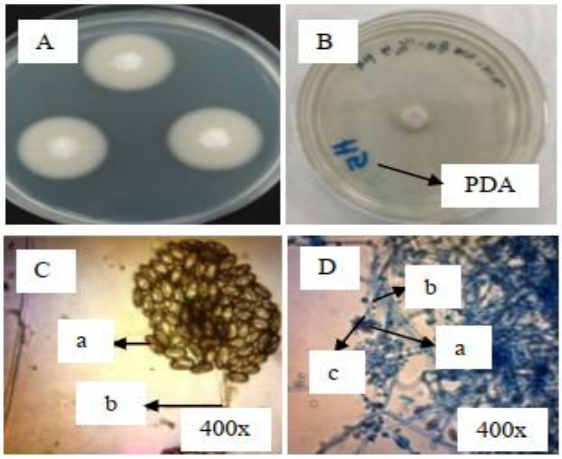

Figure 2. Macroscopic and microscopic characteristics of *Acremonium* sp. (a: conidia, b: conidiophore, c: phialid).

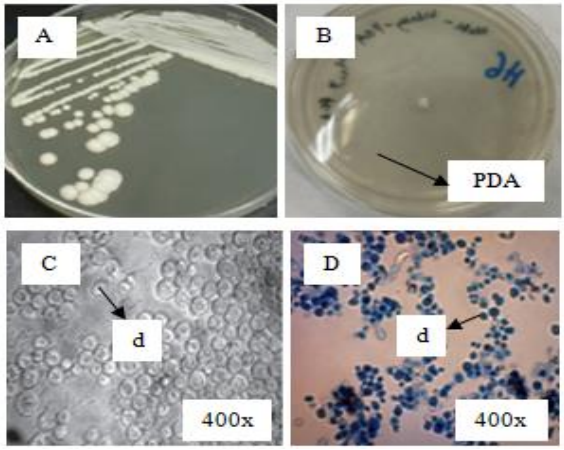

Figure 3. Macroscopic and microscopic characteristics of *Saccharomyces* sp. (d: spore).

The fourth fungus identified as *Penicillium kewense* (Kulik 1968). This fungus can be purified in PDA media. *P. kewense* have purplish-brown colonies and a slightly rough texture, while the microscopic characteristics are the round and ellipsoid conidia, the elongated phialid and the erect conidiophore (Figure 4).

Then, the *O. goramy* sample was overgrown with fungi that have white colonies and a smooth (like cotton) texture, their microscopic characteristics being the elongated threads, with some pointed ends, and the round conidia (Figure 5). These characteristics cannot identify the type of fungus, so the fungus with the isolate code HGII3 is said to be unidentified. The HGII3 fungus can be purified on PDA and PDB media.

The *O. goramy* sample was then overgrown with fungi that have white colonies with a smooth vto slightly rough and dense texture (in the middle), while the microscopic characteristics are a shape like elongated fine fibers and the round conidia (Figure 6). These characteristics cannot identify the type of fungus, so the fungus with the isolate code HGI5b is said to be unidentified. HGI5b fungi can be purified in PDA and PDB media.

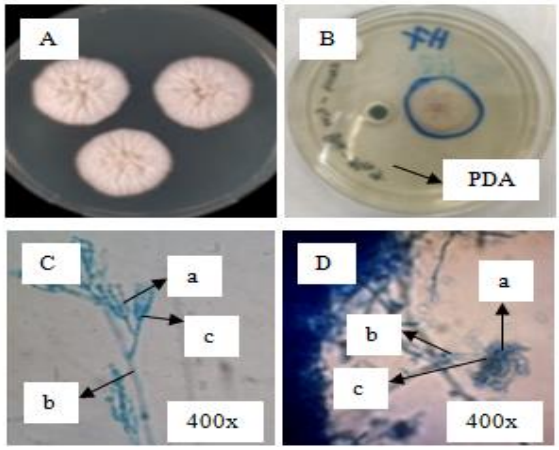

Figure 4. Macroscopic and microscopic characteristics of *Penicillium kewense* (a: conidia, b: conidiophore, c: phialid).

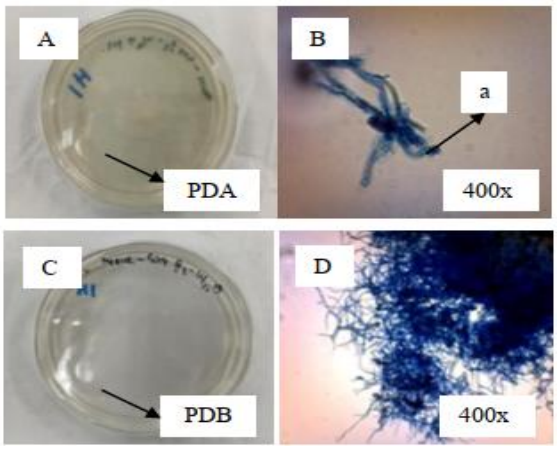

Figure 5. Macroscopic and microscopic characteristics of HGII3 fungi (a: conidia).

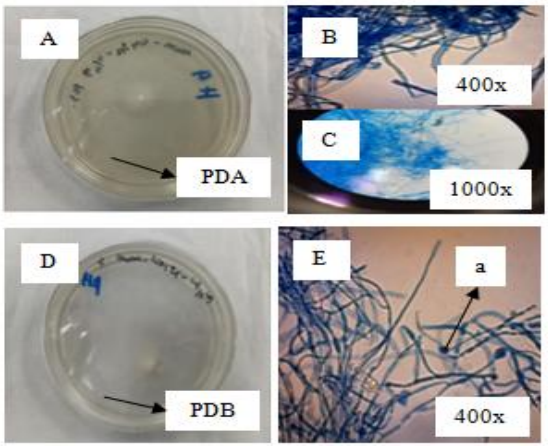

Figure 6. Macroscopic and microscopic characteristics of HGI5b fungi (a: conidia).

Then, the *O. goramy* sample was overgrown with fungi that have white colonies, a greenish center and a smooth texturewhile its microscopic characteristic is a shape like elongated fine threads (Figure 7). These characteristics cannot identify the type of fungus, so the fungus with the isolate code HGII1a is said to be unidentified. HGII1a fungi can only be purified in PDA media.

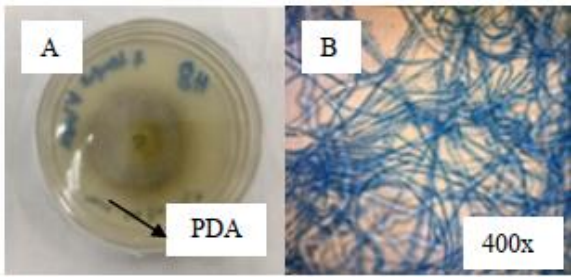

Figure 7. Macroscopic and microscopic characteristics of HGII1a fungi.

The complete data of the identification of opportunistic pathogenic fungi in *O. goramy* from Kampung Gong Badak Fish Market, Kuala Terengganu, Malaysia can be seen in Table 2.

Table 2

Identification results of opportunistic pathogenic fungi in *Osphronemus goramy*

No	*Isolate code*	*Species of fungus*
1	HGII3	Unidentfied
2	H2KPDA, HGI6	*Exophiala* sp.
3	HGI5b	Unidentfied
4	HGIII2b	*Acremonium* sp.
5	H6KPDA	*Saccharomyces* sp.
6	HGIII1a	*Penicillium kewense*
7	HGII1a	Unidentfied

Furthermore, the macroscopic and microscopic characteristics of the opportunistic pathogenic fungi that were identified in *O. goramy* from the Kampung Gong Badak Fish Market, Kuala Terengganu, Malaysia can be seen in Table 3 and Table 4.

Table 3

Macroscopic characteristics of opportunistic pathogenic fungi in *Osphronemus goramy*

No	*Species of fungus*	*Form*	*Color*	*Elevation*	*Margin*	*Surface*
1	*Exophiala* sp.	Irregular	Grayish black	Raised	Undulate	Compact
2	*Acremonium* sp.	Circular	Pale orange	Raised	Undulate	Compact
3	*Saccharomyces* sp.	Circular	White	Raised	Undulate	Smooth
4	*Penicillium kewense*	Circular	Purplish brown	Raised	Entire	Rough

Table 4

Microscopic characteristics of opportunistic pathogenic fungi in *Osphronemus goramy*

No	*Species of fungus*	*Conidia/spore*	*Conidiophore*	*Phialid*
1	*Exophiala* sp.	Round conidia	Erect conidiophores	Without phialid
2	*Acremonium* sp.	Round conidia	Erect conidiophores	Long phialid
3	*Saccharomyces* sp.	Round spores	Without conidiophores	Without phialid
4	*Penicillium kewense*	Oval conidia	Erect conidiophores	Long phialid

Protease activity test of opportunistic pathogenic fungi. Based on the results of the protease activity test in *O. goramy* from Kampung Gong Badak Fish Market, Kuala Terengganu, Malaysia, it was found that two species of fungi showed a protease enzymatic activty: *Acremonium* sp. and *P. kewense* (Figure 8). The test results of the protease enzymatic activity of opportunistic pathogenic fungi in *O. goramy* from Kampung Gong Badak Fish Market, Kuala Terengganu, Malaysia can be seen in Table 5.

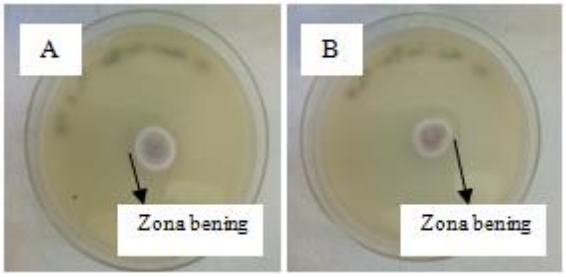

Figure 8. Protease activity test of opportunistic pathogenic fungi in *Osphronemus goramy* (A: *Acremonium* sp., B: *Penicillium kewense*).

Table 5
Protease activity test results of opportunistic pathogenic fungi in *Osphronemus goramy*

No	*Isolate code*	*Species of fungus*	*Protease activity test results*	*Protein hydrolysis index*
1	HGII3	Unidentified	Negative (-)	-
2	HGI6	*Exophiala* sp.	Negative (-)	-
3	HGI5b	Unidentified	Negative (-)	-
4	HGIII2b	*Acremonium* sp.	Positive (+)	1.44
5	H6KPDA	*Saccharomyces* sp.	Negative (-)	-
6	HGIII1a	*Penicillium kewense*	Positive (+)	1.50
7	HGII1a	Unidentified	Negative (-)	-

Discussion. It is known that the fungus is found in the gill and kidney of *O. goramy*. No fungus was found in the muscle, liver, eye and water samples. These results are in accordance with Andika et al (2014) and Zakaria et al (2020): fungi can grow in the gill and kidney of *O. goramy*. Four types of fungi were identified in the *O. goramy* sample: *Exophiala* sp., *Acremonium* sp., *Saccharomyces* sp. and *P. kewense*.

Table 6
Identification of opportunistic pathogenic fungi in *Osphronemus goramy* (in this study) compared with other studies

No	*Location*	*Species of fungus*	*Source*
1	Kuala Terengganu, Malaysia	*Exophiala* sp., *Acremonium* sp., *Saccharomyces* sp., *Penicillium kewense*	Present study
2	East Java, Indonesia	*Saprolegnia* sp., *Aphanomyces* sp.	Budiana & Rahardja (2018)
3	Central Java, Indonesia	*Aspergillus* sp.	Khumaidi & Hidayat (2018)
4	West Kalimantan, Indonesia	*Saprolegnia* sp.	Andika et al (2014)
5	Selangor, Malaysia	*Aphanomyces* sp.	Afzali et al (2013)
6	Isfahan, Iran	*Penicillium* sp., *Acremonium* sp., *Alternaria* sp.	Shahraki et al (2014)
7	Vienna, Austria	*Aphanomyces invadans*	Majeed et al (2017)

The fungi that have been identified are opportunistic pathogenic fungi, usually saprophytic and which do not cause disease in animals and humans. This fungus has the potential to cause disease if it is in a certain environment or when the host's immune condition decreases (Vázquez-González et al 2013). The first factor that causes opportunistic pathogenic fungi in *O. goramy*, is the difference in the water quality between the cultivation area and the fish market where the *O. goramy* is found. The water in the fish market in Malaysia has a temperature ranging from 28 to 36ºC, the DO ranging from 2-12 mg L^{-1} and the pH ranging from 5.5 to 8.5, while the optimum

conditions for *O. goramy*, according to Khairyah et al (2012), are: a temperature of 28-32ºC, a DO not inferior to 2 mg L^{-1}, and a pH of 6.5-8.5. Variations in the water quality at the fish market can cause stress, leading to suboptimal survival and growth, due to the *O. goramy*'s increased susceptibility to fungal attacks.

The second factor that causes the opportunistic pathogenic fungi development consists of environmental factors during the isolation process, such as air humidity and room temperature. A high humidity can increase the water absorption and fungi growth. The room temperature factor is related to the incubation temperature of 24-25ºC during the isolation and purification process, lower than the temperature at the fish market. The difference in temperature beween the fish market and incubation temperature is caused by several factors, such as the sampling time, fungus metabolism and nutrient absorption by each fungi species. Opportunistic pathogenic fungi can grow at temperatures of 25-37ºC (Vázquez-González et al 2013). The incubation temperature is as follows: for the *Exophiala* sp. it is of 25ºC for 5-21 days (Libert et al 2016), for the *Acremonium* sp. it is of 25ºC for 3-7 days (Park et al 2017), for the *Saccharomyces* sp. it is of 25ºC for 14 days (Anoop et al 2015) and for the *P. kewense* the is of 25ºC for 7 days (Houbraken et al 2012). Based on the mentioned literature, it appears that the types of opportunistic pathogenic fungi in *O. goramy* can be incubated at room temperature, resulting in variable growth times, depending on the ability of each fungi species to grow.

Based on data from several other studies (Table 6), it is known that *O. goramy* can also be attacked by other opportunistic pathogenic fungi, such as *Saprolegnia* sp., *Aphanomyces* sp., *Aspergillus* sp. and *Alternaria* sp. These fungi can be found in eggs, fins, skin, gills, eyes and water samples. This is in accordance with Andika et al (2014), which states that a suboptimal water quality has the potential to cause fungal attacks on *O. goramy* eggs. The fungal attack is accompanied by a rapid spread, especially on the skin, fins, gills and eyes of *O. goramy*. Compared with the conducted studies, opportunistic pathogenic fungi were only found in gill and kidney of *O. goramy*. The fungi found on the gill, which is a part of the respiratory system, are due to the entrance of materials that come from outside the fish's body. The gill uses mucus as a surface barrier, which is toxic and can catch microorganisms (including fungi) that enter the fish's body. Fungi found in the kidney are due to the poor water quality (Jasmanindar 2011). *O. goramy* can also ingest fungal spores with the water or feed that has been contaminated by fungi (Pinheiro et al 2018).

Opportunistic pathogenic fungi were not found in samples of other target organs, such as eye, liver and muscle in this study due to a too low density: during the isolation process, no fungi were growing on the media (Olga 2012), also due to the contamination with bacteria inhibiting the growth of fungi. Opportunistic pathogenic fungi were found neither in the water samples. The reason is that water sampling was only carried out on the surface and the water was not stirred first (when sampling), resulting in a low density of the fungus to be isolated. Stirring of the water sample should be done slowly to resuspend the sediment at the bottom (Wulandari et al 2014).

The protease activity test in this study was carried out qualitatively using skim milk mixed in PDA media. After the inoculation and incubation process, two types of fungi, *Acremonium* sp. and *P. kewense*, showed the protease enzymatic activity. This is in accordance with Jain et al (2012) and Ikram-ul-haq et al (2006) who stated that fungi of the genus *Acremonium* and *Penicillium* can show a protease activity. Skim milk contains casein as a protease substrate. The activity of protease enzymes can be seen from the hydrolysis of casein into soluble peptides and amino acids, which are then used by the metabolism of the cell growth and development (Setiawan et al 2016). The hydrolysis of casein in the media was indicated by the presence of a clear zone around the fungal colonies. The occurrence of higher proteolytic activity is indicated by the wider clear zone formed (Ikhsanudin et al 2019).

The protein hydrolysis index was calculated by comparing the diameter of the clear zone with the diameter of the fungal isolate colonies (Hastuti et al 2017). The difference of the Protein Hydrolysis Index values in each fungal isolate was due to the different ability of each fungus to produce protease enzymes. *P. kewense* has a Protein Hydrolysis Index of 1.50 while *Acremonium* sp. has a protein hydrolysis index of 1.44.

According to Ahmad et al (2013), the category of protein hydrolysis index is low if lower than 2.1, moderate if ranging from 2.1 to 3.1 and high if greater than 3.1. The Protein Hydrolysis Index of both the mentioned opportunistic pathogenic fungi was in the low category (<2.1).

The production of protease enzymes by fungi can affect their infectious potential towards their hosts. Protease enzymes can help fungi degrading the main components that make up the cuticle of their host. *Acremonium* sp. and *P. kewense* had a low Protein Hydrolysis Index, meaning that both fungi had a low ability to produce protease enzymes. The lower the protease enzyme produced, the lower the degree of pathogenicity (virulence) of the fungus (Supiyanto et al 2019). With a low ability to produce protease enzymes, *Acremonium* sp. and *P. kewense* can be said to have no potential to cause death or even disease in *O. goramy*.

Table 7

Protease activity test of opportunistic pathogen fungi compared to other studies

No	*Species of fungus*	*Enzyme*	*Source*
1	*Acremonium* sp., *Penicillium kewense*	Protease	Present study
2	*Exophiala* sp.	Lipase, DNAse, α-Amylase, Pectinase, Cellulase, Protease	Mythili et al (2014)
3	*Acremonium* sp.	Protease	Abd-ElAzeem et al (2019)
4	*Saccharomyces* sp.	Amylase, Protease, Pectinase	Jayalakshmi & Umamaheswari (2017)
5	*Penicillium* sp.	Protease	Ikramul-haq et al (2006)

Based on the data in Table 7, it appears that the types of fungi identified in this study can also produce enzymes other than protease enzymes, such as lipase, DNAse, amylase, α-amylase, pectinase, and cellulase enzymes. These enzymes have different characters and functions. Lipase enzyme are usually secreted by fungi to degrade components of the plasma membrane of their host, for using it as a source of nutrition. The DNAse enzyme has the ability to cut DNA into small fragments, destroying it, so the presence of this enzyme can reduce the quantity of DNA extract produced by fungi (Marwayana 2015). The amylase enzyme can hydrolyze starch substrates into simpler carbohydrate compounds such as maltose and glucose. Lipase degradation products can be a source of nutrition during the fungal colonization. Amylase enzymes produced by fungi are more stable than enzymes produced by bacteria. Amylase enzymes can be classified according to the way they cleave the glysosidic bonds; α-amylase is an endo-amylase that hydrolyzes alpha 1,4-glycosidic bonds, randomly generating dextrins, oligosaccharides and monosaccharides. Pectinase enzymes can be induced to the fungi in the presence of a pectin substrate. This enzyme is very important in the process of fungal phytopathology. Finally, cellulase enzymes are grouped based on their specific activity on the substrate: endoglucanase, cellobiohydrolase, and exoglucohydrolase. These three enzymes work together in breaking down cellulose. Fungi that produce proteases and pectinases are usually potentially pathogenic. Fungi will be mutualistic and sometimes they become saprophytes when they produce cellulase enzymes (Susilowati et al 2020).

Based on Table 7, it is known that *Exophiala* sp. and *Saccharomyces* sp. should be able to produce protease enzymes. In this study, the absence of protease enzyme activity in these fungus could be caused by the short testing time, so that the proteolytic activity produced was not significant. The short test time was accompanied by a slower fungal growth time, so that only a few fungi grew on the media, and these fungi did not show the expected proteolytic activity. Other factors, such as temperature, pH, as well as the substrate and enzyme concentrations, can also affect the activity of protease enzymes (Sajuthi et al 2010).

Based on the results of the current study, the *O. goramy* sample specimens were healthy: the fish had a bright color, the body was clean from parasites, the organs were

complete and undamaged and their movement was agile (Syafar et al 2017). The seven fungal species identified did not cause disease in the *O. goramy* samples. The reason is that the fungi identified had a low density and the potential to produce toxic metabolites (mycotoxins) only in low amounts and under certain conditions (Noveriza 2008). Mycotoxins can interfere with the health of the host causing various forms of clinical and pathological changes. However, the presence of fungal growth in fish does not always cause mycotoxins production (Kumaji 2018).

Conclusions. Seven species of opportunistic pathogenic fungiwere isolated from the gill and kidney of *O. goramy*: three species were undentified and four other species were identified as *Exophiala* sp., *Acremonium* sp., *Saccharomyces* sp. and *P. kewense*. Two types of fungus, *Acremonium* sp. and *P. kewense* showed a protease enzymatic activity, with the protein hydrolysis index of 1.44 and 1.50, respectively, which correspond to the low category (<2.1).

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References

Abd-ElAzeem E. M., El-Medany W. A. Z., Sabry H. M., 2019 Biological activities of spores and metabolites of some fungal isolates on certain aspects of the spiny bollworms *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae). Egyptian Journal of Bioloigical Pest Control 29(90):1-7.

Afzali S. F., Abdul-Rahim H., Sabri J., 2013 Isolation and identification of *Aphanomyces* species from natural water bodies and fish farms in Selangor, Malaysia. Malaysian Applied Biology 42:21–31.

Ahmad B., Nigar S., Shah S. S. A., Bashir S., Ali J., Yousaf S., Bangash J. A., 2013 Isolation and identification of cellulose degrading bacteria from municipal waste and their screening for potential antimicrobial activity. World Applied Sciences Journal 27(11):1420-1426.

Akmalasari I., Purwati E. S., Dewi S., 2013 [Isolation and identification of endophytic fungi from mangosteen plant (*Garcinia mangostana* L.)]. Biosfera 30(2):82–89. [In Indonesian].

Andika H., Dewantoro E., Rahardjo E. I., 2014 [Soaking egg carp (*Osphronemus goramy*) with extract of meniran (*Phyllanthus niruri* L) as anti fungus]. Jurnal Ruaya: Jurnal Penelitian dan Kajian Ilmu Perikanan dan Kelautan 1(1):71-76. [In Indonesian].

Andlid T., Blomberg L., Gustafsson L., Blomberg A., 1999 Characterization of *Saccharomyces cerevisiae* CBS 7764 isolated from rainbow trout intestine. Systematic and Applied Microbiology 22(1):145-155.

Anoop V., Rotaru S., Shwed P. S., Tayabali A. F., Arvanitakis G., 2015 Review of current methods for characterizing virulence and pathogenicity potential of industrial *Saccharomyces cerevisiae* strains towards humans. FEMS Yeast Research 15(6):1-12.

Brown S. P., Conforth D. M., Mideo N., 2012 Evolution of virulence in opportunistic pathogens: generalism, plasticity, and control. Trends in Microbiology 20(7):336-342.

Budiana, Rahardja B. S., 2018 [Goramy (*Osphronemus goramy*) breeding techniques at Fish Seed Center Ngoro, Jombang]. Journal of Aquaculture and Fish Health 7(3):90-97. [In Indonesian].

Domsch K. H., Gams W., Anderson T. H., 2007 Compendium of soil fungi. 2nd edition. IHW-Verlag, Germany, 672 p.

Du N. N., Starr P., 2010 Ornamental fish farms flourish in Viet Nam with both exotic and native species. MRC Catch and Culture 16(1):14-18.

Gaskins J. E., Cheung P. J., 1986 *Exophiala pisciphila*: a study of its development. Mycopathologia 93(3):173-184.

Hardaningsih I., 2018 [Gourami cultivation for food security and eradication possibilities in rural areas: opportunities and challenges]. Makalah pada Seminar Nasional Perikanan ke XV, Yogyakarta, Indonesia, 19 p. [In Indonesian].
Hastuti U. S., Nugraheni F. S. A., Asna P. M. A., 2017 [Identification and determination of protein hydrolysis index of proteolytic bacteria from Margomulyo mangrove soil, Balikpapan]. Proceeding Biology Education Conference 14(1):265-270. [In Indonesian].
Hastuti U. S., Hapsari L., Khasanah H. N., 2015 Isolation and identification of contaminant molds on pumpkin candy from Sumbawa Besar. National Seminar XII Pendidikan Biologi FKIP UNS 843-848.
Houbraken J., Frisvad J. C., Seifert K. A., Overy D. P., Tuthill D. M., Valdez J. G., Samson R. A., 2012 New penicillin-producing *Penicillium* species and an overview of section *Chrysogena*. Persoonia 29:78–100.
Ikhsanudin A., Rosa E., Ekowati C. N., 2019 Proteolytic activity of the entomopathogenic fungi (*Penicillium* sp.) of cockroaches (*Periplaneta americana*). Jurnal Ilmiah Biologi Eksperimen dan Keanekragaman Hayati 6(2):81-84.
Ikram-ul-haq, Mukhtar H., Umber H., 2006 Production of protease by *Penicillium chrysogenum* through optimization of environmental conditions. Journal of Agriculture and Social Sciences 2(1):1813–2235.
Inger R. F., Chin P. K., 1962 The fresh-water fishes of North Borneo. Fieldiana: Zoology 45:1-268.
Ishak N. A. B., 2010 The effectiveness of using effective microorganisms (EM) in fish market wastewater. PhD thesis, Faculty of Civil Engineering and Earth Resources, Universiti Malaysia Pahang, 81 p.
Ito H., Abu M. Y., 1985 Study of microflora in malaysian dried fishes and their decontamination by gamma-irradiation. Agricultural and Biological Chemistry 49(4):1047-1051.
Jain P., Aggarwal V., Sharma A., Pundir R. K., 2012 Isolation production and partial purification of protease from an endophytic *Acremonium* sp. Journal of Agricultural Technology 8(6):1979-1989.
Jasmanindar Y., 2011 [Prevalence of parasites and diseases of cultured freshwater fish in city/ regency of Kupang]. Bionatura 13(1):25-30. [In Indonesian].
Jayalakshmi N., Umamaheswari G., 2017 Production and optimization of amylase enzyme from *Saccharomyces cerevisiae* by mangrove environ. International Journal of Science and Research 6(5):2524-2526.
Khairyah U., Kusdarwati R., Kismiyati, 2012 [Identification and the prevalence of fungal goramy (*Osphronemus goramy*) in Ngrajek Village, Mungkid Sub-District, Magelang District, Central Java]. Journal of Aquaculture and Fish Health 1(2):1-7. [In Indonesian].
Khumaidi A., Hidayat A., 2018 [Identification of causes of mass death of gurami fish (*Osphronemus goramy*) in Gurami Fish Cultivation Sentra, Desa Beji, Kedung Banteng District, Banyumas District, Central Java]. Journal of Aquaculture Science 3(2):145-153. [In Indonesian].
Kulik M. M., 1986 A compilation of descriptions of new *Penicillium* species. Agriculture Handbook No. 351. Agricultural Research Service. United States Department of Agriculture, 80 p.
Kumaji S. S., 2018 [Identification of contaminating molds in smoked skipjack tuna (*Katsuwonus pelamis*) at the Central Market of Gorontalo City]. Jurnal Entropi 13(1):109-114. [In Indonesian].
Kurtzman, Fell J. W., 1998 The yeasts: a taxonomic study. 4th edition. Elsevier Science Publishers B. V. Amsterdam, pp. 72-102.
Kusdarwati R., Sudarno, Hapsari A., 2016 [Isolation and indentification of fungi on the gold fish (*Carassius auratus*) in the Fish Market Gunung Sari Surabaya East Java]. Jurnal Ilmiah Perikanan dan Kelautan 8(1):1-15. [In Indonesian].
Libert X., Chasseur C., Packeu A., Bureau F., Roosens N. H., De Keersmaecker S. J. C., 2016 A molecular approach for the rapid, selective and sensitive detection of *Exophiala jeanselmei* in environmental samples: development and performance

assessment of a real-time PCR assay. Applied Microbiology and Biotechnology 100:1377–1392.
Majeed M., Kumar G., Schlosser S., El-Matbouli M., Saleh M., 2017 In vitro investigations on extracellular proteins secreted by *Aphanomyces invadans*, the causative agent of epizootic ulcerative syndrome. Acta Veterinaria Scandinavica 59(78):1-9.
Marwayana O. N., 2015 [Extraction of deoxyribonucleic acid (DNA) from muscle tissue samples]. Oseana 40(2):1-9. [In Indonesian].
McGinnis M. R., 1980 Laboratory handbook of medical mycology. Academic Press, 434 p.
Mythili A., Singh Y. R. B., Priya R., Hassan A. S., Manikandan P., Panneerselvam K., Narendran V., Shobana C. S., 2014 In vitro and comparative study on the extracellular enzyme activity of molds isolated from keratomycosis and soil. International Journal of Ophthalmology 7(5):778-784.
Nevalainen H., Kautto L., Te'o J., 2014 Methods for isolation and cultivation of filamentous fungi. Molecular Biology 1096:3-16.
Noveriza R., 2008 [Contamination of fungal and mycotoxins on medicinal plants]. Perspektif 7(1):35-46. [In Indonesian].
Nugroho E., 2012 [*Endang pamularsih* as a superior gourami]. Media Akuakultur 7(2): 99-102. [In Indonesian].
Olga, 2012 [The pathogenicity of *Aeromonas hydrophila* ASB01 on snakehead (*Ophicephalus striatus*)]. Sains Akuatik 14(1):33-39. [In Indonesian].
Park S. W., Nguyen T. T., Lee H. B., 2017 Characterization of two species of *Acremonium* (unrecorded in Korea) from soil samples: A. *variecolor* and A. *Persicinum*. Research Article of Mycobiology 45(4):353-361.
Pinheiro R. E. E., Rodrigues A. M. D., de O. Santos J. T., de A. Costa J., Pereyra C. M., Torres A. M., Rosa C. A., de O. Santos A. R., Muratori M. C. S., 2018 Occurrence and diversity of yeast species isolated from fish feed and tambatinga gut. Latin American Journal of Aquatic Research 46(4):837-842.
Pinzari F., Montanari M., Michaelsen A., Piñar G., 2010 Analytical protocols for the assessment of biological damage in historical documents. Coalition, pp. 6-13.
Sajuthi D., Suparto I., Yanti, Praira W., 2010 [Purification and characterization of fibrinolytic protease enzymes from mushroom extracts]. Makara Science 14(2):145-150. [In Indonesian].
Setiawan A., Arimurti S., Senjarini K., Sutoyo, 2016 [Proteolytic and fibrinolytic activity of bacterial isolates from the Papuma Beach on Jember District]. Berkala Sainstek 4(1):1-4. [In Indonesian].
Shahraki M. M., Asgari M. R., Khamesipour F., Raissy M., 2014 [Prevalence of *Argulus foliaceus* and fungal infections in some ornamental fishes [discus *(Symphysodon discus)*, dwarf gourami *(Trichogaster lalius)* and guppy *(Poecilia reticulata)*] in Isfahan City of Iran]. Kafkas Universitesi Veteriner Fakultesi Dergisi 20 (5): 817-820. [In Indonesian].
Supiyanto, Rosa E., Irawan B., Nukmal N., 2019 [Isolation and pathogenicity test of entomopathogenic fungi isolates against the adult stage of the *Aedes aegypti* mosquito.] Jurnal Biologi Papua 11(1):33-41. [In Indonesian].
Susilowati D. N., Setiyani A. D., Radiastuti N., Sofiana I., Suryadi Y., 2020 [Diversity of extracellular enzymes produced by endophytic fungus originated from *Centella asiatica* (L.) urban]. Industrial Crops Research Journal 26(2):78-91. [In Indonesian].
Syafar L. A., Mahasri G., Rantam F. A., 2017 Blood description, parasite infestation and survival rate of carp (*Cyprinus carpio*) which is exposed by spore protein *Myxobolus koi* on rearing pond as immunostimulan material. Jurnal Biosains Pascasarjana 19(2):158-179.
Vázquez-González D., Perusquía-Ortiz A. M., Hundeiker M., Bonifaz A., 2013 Opportunistic yeast infections: candidiasis, cryptococcosis, trichosporonosis and geotrichosis. Journal of The German Society of Dermatology 11(5):381-393.
Widiastutik N., Alami N. H., 2014 [Isolation and identification of yeast from the rhizosphere Rhizophora mucronata Wonorejo]. Jurnal Sains dan Seni (Pomits) 3(1):2337-3520. [In Indonesian].

Wulandari N., Sukanto, Widyastuti E., 2014 [Effect of giving effective productive plus (MEP+) microbes on tilapia culture media fed fermentative feed on the density of lactic acid bacteria]. Scripta Biologica 1(1):61-65. [In Indonesian].
Yuniati R., Nugroho T. T., Puspita F., 2015 [Protease enzymes activity test of Bacillus sp. strain from Riau]. Jurnal Online Mahasiswa Fakultas Matematika dan Ilmu Pengetahuan Alam 1(2):116-122. [In Indonesian].
Yusoff A., 2015 Status of resource management and aquaculture in Malaysia. Proceedings. International Workshop on Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia, pp. 53-65.
Zakaria K., Teet S. E., Hamzah N. H., Aznan A. S., Manaf M. T. A., Ibrahim W. N. W., Leong L. K., Iberahim N. A., Musa N., Abdulrazzak L., Daud H. M., Taib M., Hatai K., John A., Jalal K. C. A., Sheikh H. I., Musa N., 2020 Isolation and identification of fungi associated with diseased freshwater fishes in Terengganu, Malaysia. Songklanakarin Journal of Science and Technology, pp. 3-28.

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