IJES
Indonesian Journal of Environmental Sustainability

Published by : Center For Environmental Studies UIN Ar-Raniry Banda Aceh

IDENTIFICATION OF FUNGI IN CAMPUS TOILET BATHROOM WATER

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Received : 12 February 2023 Accepted : 12 March 2023 Published : 27 June 2023

ABSTRACT

The recognition of the health risks associated with fungal contamination in water is increasingly important in terms of microbiological safety and water quality. Fungus is one type of microorganism that can cause disease in humans. As cosmopolitan living things, fungi can be found anywhere close to human existence, be it in water, soil, air, or even in the human body. The purpose of this study was to determine the total number of colonies and the characteristics of pathogenic fungi in bathroom water at UIN Ar-Raniry campus public toilet facilities. This study used bathroom water samples taken at 5 different points. Water samples were taken as much as 0.5 ml and then isolated into Potato Dextrose Agar (PDA) media using the spread plate method. After 7 days of incubation, the total number of colonies was counted and the identification of the fungi was carried out to determine the macroscopic and microscopic characteristics of the fungi. The results of the study of fungal isolation on PDA media were the total colonies obtained from 5 different locations, namely 60 colonies, 47 colonies, 25 colonies, 29 colonies and 46 colonies. The identification results of 31 pure isolates found species namely Aspergillus sp., Aspergillus niger, A. fumigatus, A. flavus, Penicillium sp., and Mucor sp.

Keywords : Water Contamination, Fungal Identification

ABSTRAK

Pengenalan resiko kesehatan yang berhubungan dengan kontaminasi jamur dalam air semakin penting dalam keamanan mikrobiologis dan kualitas air. Jamur adalah salah satu jenis mikroorganisme yang dapat menimbulkan penyakit pada manusia. Sebagai makhluk hidup kosmopolitan, jamur dapat ditemukan dimana saja dekat dengan keberadaan manusia, baik itu di air, tanah, udara, bahkan di tubuh manusia. Tujuan penelitian ini adalah untuk mengetahui jumlah total koloni dan karakteristik jamur patogen pada air kamar mandi di fasilitas toilet umum kampus UIN Ar-Raniry. Penelitian ini menggunakan sampel air kamar mandi yang diambil di 5 titik berbeda. Sampel air diambil sebanyak 0,5 ml lalu diisolasi ke dalam media Potato Dextrose Agar (PDA) dengan metode cawan sebar. Setelah diinkubasi selama 7 hari kemudian dilakukan perhitungan jumlah total koloni dan identifikasi jamur untuk mengetahui karakteristik makroskopis dan mikroskopis jamur. Hasil penelitian isolasi jamur pada media PDA yaitu total koloni yang diperoleh masing-masing dari 5 lokasi berbeda yaitu 60 koloni, 47 koloni, 25 koloni, 29 koloni dan 46 koloni. Hasil identifikasi dari 31 isolat murni ditemukan spesies yaitu Aspergillus sp., Aspergillus niger, A. fumigatus, A. flavus, Penicillium sp.,dan Mucor sp.

Kata kunci : *Kontaminasi Air, Identifikasi Jamur*

Introduction

Clean toilet facilities and water hygiene are important aspects of health. Without adequate toilet facilities and water hygiene, human lives are at serious health risk. Toilet and water hygiene are functions of sanitation and are important indicators of public health. Clean toilets refer to the type of toilet used, clean conditions to prevent the transmission of infectious diseases between community members. While water hygiene refers to the source of water, as well as the handling of water sources to improve optimal public health (Akingbade, 2019). Ensuring human health is related to several factors, including water quality. To be in an ideal state for consumption, water must meet microbiological standards that serve as indicators of pollution (Arroyo, 2020).

Fungi are one type of microorganism that can cause disease in humans. As cosmopolitan living beings, fungi can be found anywhere close to human existence, be it in water, soil, air, and even in the human body (Hasanah, 2017). The presence of fungi in water is often overlooked because their significance is limited to taste and odor (Shittu, 2022). Species found in natural aquatic environments are most often represented by microscopic fungi from the classes Chytridiomycetes, Oomycetes, Trichomycetes, and Mucoromycetes. Of the many estimated fungal species, only 3,000-4,000 are classified as aquatic fungi (Goralska, 2020).

Water is one of the sources of disease spread so it is necessary to monitor water quality. Many diseases are transmitted through water from bacterial, virus, parasitic and fungal contamination. Pathogenic fungi can grow easily in places that are humid and have a tropical climate. Pathogenic fungi usually live in the wild such as in soil, organic debris and water, so water is easily contaminated by fungi. Poorly maintained water sources can also cause mold contamination. In addition, the lack of cleaning of water tanks allows fungi to grow and develop in the water so that the water will become a source of infection for those who use it (Irawan, 2019).

Methods

The tools used are beaker glass, stirring rod, 10 ml sample bottle, petri dish, erlenmeyer, autoclave, oven, microscope, drop pipette, object glass, cover glass, bunsen, round and straight ose needle, gloves, mask, analytical balance, media spoon, measuring cup, micropipette and incubator. While the materials used in this study are bathroom water, distilled water, 70% alcohol, Lactophenol Cotton Blue, plastic wrap, aluminum, distilled water, chloramphenicol and Potato Dextrose Agar (PDA) media.

Sterilization of tools used in research such as Petri dishes using an oven with a temperature of 170ºC for 1 hour. While the materials, especially PDA media, were sterilized using an autoclave at 121ºC for 15 minutes.

Samples were taken from bathroom water in public toilet facilities at the Faculty of Science and Technology, Faculty of Tarbiyah and Keguruan, Fathun Qarib mosque of UIN Ar-Raniry, Ma'had Putri UIN Ar-Raniry and Wisma UIN Ar-Raniry. Samples were taken in the amount of 1 sample in each place as much as 10 ml and then put in a sterile bottle.

Water samples that have been put into a sterile bottle as much as 10 ml are taken as much as 0.5 ml using a micropipette and then put in PDA media that has been compacted beforehand and spread evenly using an L rod. Each water sample is spread in 5 petri dishes. Then incubated for 5-7 days. After the fungus grows then purified. Then the number of fungal colonies was counted directly. Identification of pathogenic fungi is done macroscopically and microscopically.

Macroscopic identification is carried out by observing the growth of cultures on PDA media by paying attention to morphological characteristics such as colony shape, colony surface, color, texture, odor and consistency of colonies. Microscopic identification is carried out by direct observation under a microscope with 10x and 40x magnification to clarify the shape of the fungus (Asmarani, 2019). Microscopic identification is also needed to see the structure of hyphae and spores in fungi (Suryani, 2020).

Data analysis was carried out descriptively. After identification, the data obtained were then described in the form of tables and pictures. Fungal identification uses the microscopic fungal identification book by Alexopoulus et al. (1996), taking into account the determination key and adjusted to the existing classification according to the taxon order (Karwati, 2019). Calculation of the number of fungal colonies was carried out by directly counting the total number of fungal colonies in each Petri dish.

Results and Discussion

The calculation of total fungal colonies is done by directly counting the total number of colonies that grow in the Petri dish. The results of the total colony count can be seen in Table 1 below:

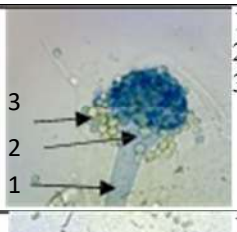
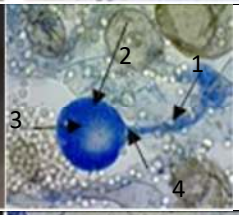
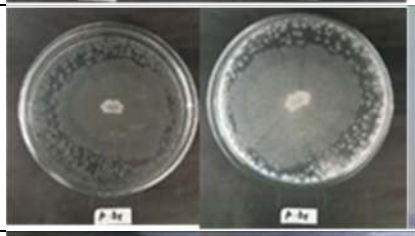
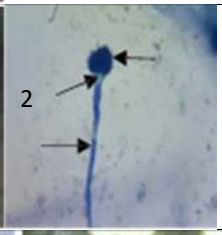
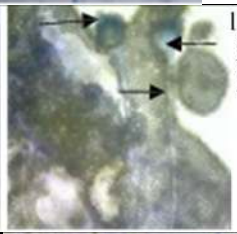
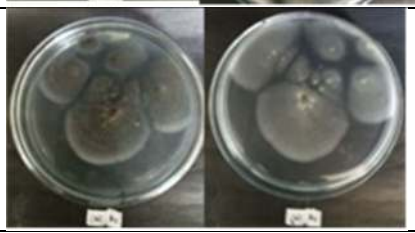
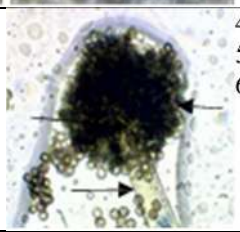
Table 1. Total number of mold colonies of bathroom water samples.

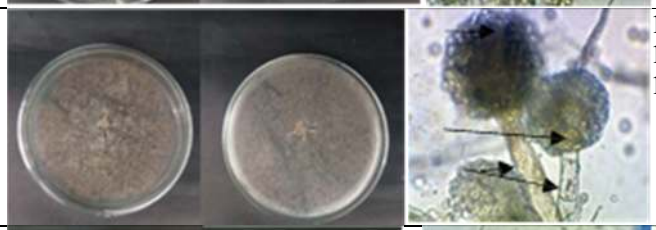
Point	Sample	Number of isolates	Total colonies	Average
1.	UIN Ar-Raniry Princess Dormitory	5 isolates	60 colonies	12 colonies
2.	Wisma UIN Ar-Raniry	5 isolates	47 colonies	9.4 colonies
3.	UIN Ar-Raniry Mosque	5 isolates	25 colonies	5 colonies
4.	Faculty of Tarbiyah & Keguruan	5 isolates	29 colonies	5.8 colonies
5.	Faculty of Science & Technology	5 isolates	46 colonies	9.2 colonies

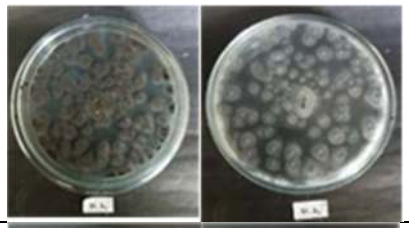
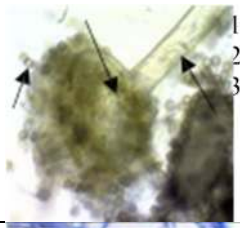
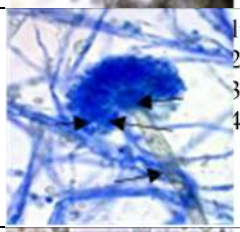
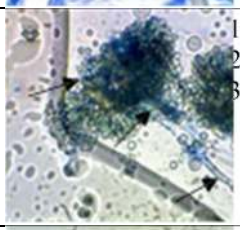
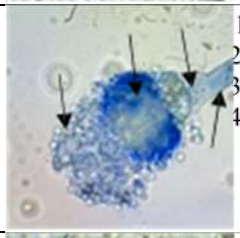
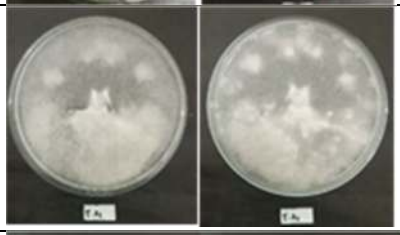
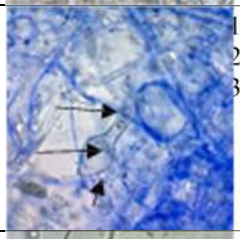
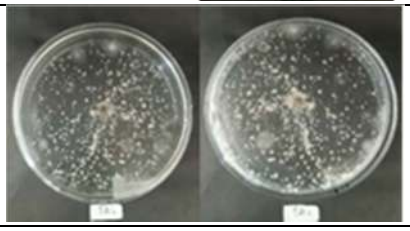
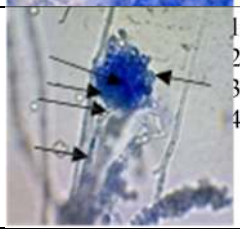
Based on the results of isolation of pathogenic fungi in bathroom water samples of public toilet facilities on the UIN Ar-Raniry campus, 31 isolates of fungal colonies were obtained. Pictures of the results of isolation of pathogenic fungi can be seen in Table 2 below:

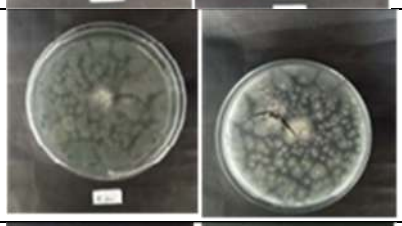
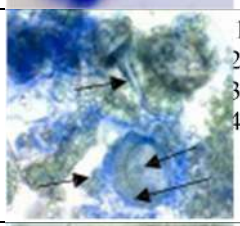
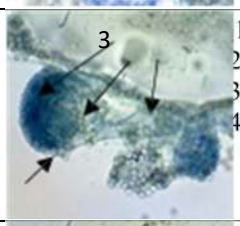
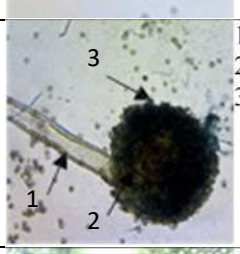
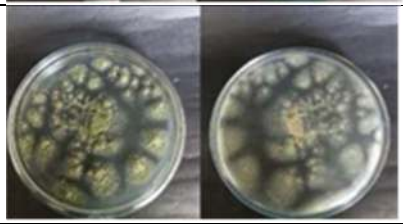
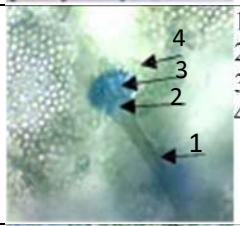
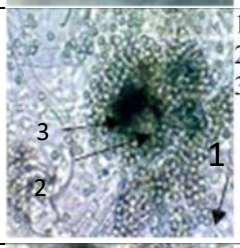
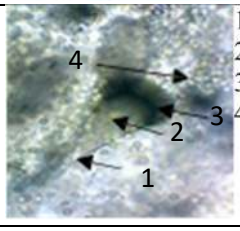
Table 2. Pictures of isolation results of pathogenic fungi in bathroom water

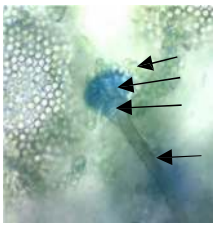
Macroscopic

No	Isolate Code	Upper colony	Flip the colony	Microscopis	Description
1	AA1				1. Conidiophore 2. Vesicles 3. Conidia
2	AA2				1. Condiophore 2. Vesicles 3. Conidia
3	AA3				1. Sporangiophore 2. Sporangium 3. sporangiospore 4. columells
4	AA4				1. Conidiophor e 2. Vesicles
5	AA5				1. Conidiophore 2. Vesicles 3. Conidia
6	WA1				1. Conidiophore 2. Vesicles 3. Conidia
7	WA2				4. Conidiophore 5. Vesicles 6. Conidia

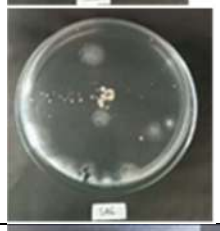
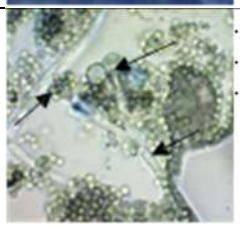
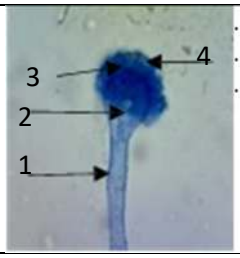
8	WA3		7. Conidiophore 8. Vesicles 9. Fialid 10. Conidia
9	WA4		11. Conidiophore 12. Vesicles 13. Conidia
10	WA5		14. Conidiophore 15. Vesicles 16. Fialid 17. Conidia
11	WA6		. Conidiophore 2. Vesicles 3. Fialid 4. Conidia
12	WA7		1. Conidiophores 2. Vesicles
13	WA8		1. Konodiofor 2. Conidia
14	MA1		. Sporangiophore . Sporangium . Sporangispores

15	MA2			1. Conidiophores 2. Vesicles 3. Conidia
16	MA3			1. Conidiophores 2. Vesicles 3. Fialid 4. Conidia
17	MA4			1. Conidiophores 2. Vesicles 3. Conidia
18	MA5			1. Conidiophores 2. Vesicles 3. Fialid 4. Conidia
19	MA6			1. Conidiophores 2. Vesicles 3. Conidia
20	TA1			1. Conidiophores 2. Vesicles 3. Conidia
21	TA2			1. Conidiophores 2. Vesicles 3. Fialid 4. Conidia

22	TA3			1. Conidiophores 2. Vesicles 3. Conidia
23	TA4			1. Conidiophores 2. Vesicles 3. Fialid 4. Conidia
24	TA5			1. Conidiophores 2. Vesicles 3. Fialid 4. Conidia
25	SA1			1. Conidiophores 2. Vesicles 3. Conidia
26	SA2			1. Conidiophores 2. Vesicles 3. Fialid 4. Conidia
27	SA3			1. Conidiophores 2. Vesicles 3. Conidia
28	SA4			1. Conidiophores 2. Vesicles 3. Fialid 4. Conidia



1

29	SA5			. Sporangiophore . Kolumela . Sporangiospore s . Sporangium
30	SA6	 		. Conidiophores . Vesicles . Conidia
31	SA7			. Conidiophores . Vesicles . Fialid

Fungal isolates that have been obtained are then macroscopically observed by looking at morphological characteristics such as colony shape, colony edges, color, elevation and colony surface. The observation results can be seen in Table 3 below:

Table 3. Morphological Characteristics of Pathogenic Fungal Isolates of Bathroom Water Samples of Public Toilet Facilities at UIN Ar-Raniry Campus

No.	Isolate Code	Colony Shape	Colony Edge	Colony Color	Elevation	Surface
1	AA1	Irregular	Notched	Light green	raised	rough
2	AA2	Concentric	Irregular	Black	flat	rough
3	AA3	Irregular	Irregular	Chocolate	raised	rough
4	AA4	Irregular	Irregular and diffuse	White	Irregular	like cotton
5	AA5	Irregular	Irregular and diffuse	Dark green	flat	rough
6	WA1	Irregular	Irregular and diffuse	Light green	flat	rough
7	WA2	Concentric	Irregular	black	flat	rough
8	WA3	Irregular	Irregular and diffuse	Dark green	flat	rough

9	WA4	Irregular	Irregular and diffuse	Chocolate	raised	rough
10	WA5	Irregular	Irregular and diffuse	Light green	raised	rough
11	WA6	Irregular	Irregular and diffuse	Dark green	flat	rough
12	WA7	Irregular	Irregular and diffuse	white	Irregular	like cotton
13	WA8	Round		Dark green With white edges	raised	smooth
14	MA1	Irregular	Irregular and diffuse	white	Irregular	like cotton
15	MA2	Concentric	Irregular	Black	flat	rough
16	MA3	Irregular	Irregular and diffuse	Light green	raised	rough
17	MA4	Round	flat	Dark green With white edges	embossed (umbonate)	smooth
18	MA5	Irregular	Irregular and diffuse	Dark green	flat	rough
19	MA6	Irregular	Irregular and diffuse	Chocolate	raised	rough
20	TA1	Irregular	Irregular and diffuse	White	Irregular	like cotton
21	TA2	Round	flat	Dark green with white edges	embossed (umbonate)	Velvety smooth
22	TA3	Concentric	Irregular	Black	flat	rough
23	TA4	Irregular	Irregular and diffuse	Chocolate	raised	rough
24	TA5	Irregular	Irregular and diffuse	Dark green	flat	rough
25	SA1	Concentric	Irregular	Black	flat	rough
26	SA1	Irregular	Irregular		raised	rough
27	SA1	Irregular	Irregular and diffuse	Dark green	flat	rough
28	SA1	Irregular	Irregular and diffuse	Dark green	flat	rough
29	SA1	Irregular	Irregular and diffuse	White	raised	like cotton
30	SA1	Round	flat	Dark green with white edges	embossed (umbonate)	Velvety smooth
31	SA1	Irregular	Irregular and diffuse	Brownish yellow	raised	rough

Discussion

Based on the isolation results of pathogenic fungi in bathroom water samples, 7 different fungal species were found. The results of identification with the microscopic fungal identification book by Alexopoulus et al., (1996) and matched with a number of journal references obtained pathogenic fungal species, including *Aspergillus sp*., *Aspergillus sp*. 1, A. *niger*, A. *fumigatus*, A. *flavus*,

Penicillium sp. and *Mucor sp*. isolates AA1, MA3, WA5 and MA5 have colonies with irregular shapes with edges, and Mucor sp. Isolates AA1, MA3, WA5 and MA5 have colonies with irregular shapes with irregular and diffuse edges, raised elevations, rough colony surfaces and appear to be light green on top of the colonies and pale green to dark green behind the colonies, except for isolate SA7 which is yellowish brown so it is identified as *Aspergillus sp*. 1. 1. *Aspergillus sp* seen under a microscope has parts, namely conidiophores that are perpendicular, round vesicles, also have phialids and conidia. This is in accordance with the description of Abbas *et al*., (2021), which states that Aspergillus sp colonies usually grow quickly, are white, yellow to yellowish brown, black or green. Most have conidiophores that are erect and feel dense. Vesicles, phialids and metulae (if present) will form conidia heads. Fungi of the genus *Aspergillus* are causative agents of *aspergillosis*, which is an infection of the lungs (Hasanah, 2017). Images of *Aspergillus sp* colonies can be seen in Figure 1 below:

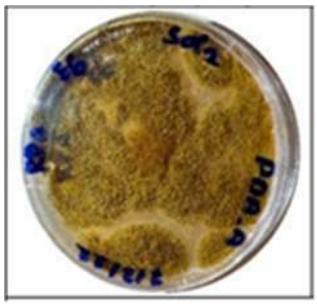


Figure 1. Colonies of the fungus *Aspergillus* sp. (Abouamama et al., 2023)

Isolates AA2, WA2, MA2, TA3, and SA1 each have the same morphological characteristics, namely concentric colony shapes, irregular colony edges, flat elevations, rough colony surfaces, and black colonies and behind pale white colonies. Microscopic examination found brown conidiophores, round vesicles, and black conidia. Based on these characteristics, this fungal colony belongs to the *Aspergillus niger* species. *The* results of these observations are in accordance with research conducted by Odrina (2023), which states that A. *niger* has black to brownish colonies and round conidia with a transparent black color and cracks when the colony is old, and has round or semi-round vesicles. Pictures of A. *niger* fungal colonies can be seen in Figure 2 below:

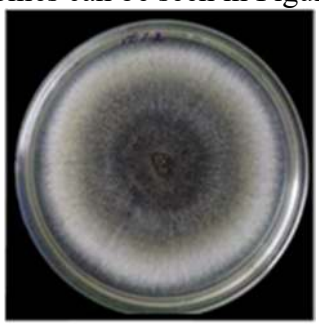


Figure 2. Colony of *Aspergillus niger* fungus (Arifah et al., 2023

DOI: https://doi.org/10.22373/ijes.v1i1.4143

The results of observations on isolates, WA3, WA6, TA5, SA3, AA5 and SA4 have irregular colony shapes, irregular and spreading colony edges, *flat* elevations, rough surfaces with dark green colony tops and white colony backs. Microscopic observation shows the parts that attack the colonies of the fungus *Aspergillus fumigatus*, in accordance with the results of research by Azzahra et al., (2020) which states that A. *fumigatus* has bluish green conidia & blue vesicles and round conidia shape. Pictures of A. *fumigatus colonies* can be seen in Figure 3 below:

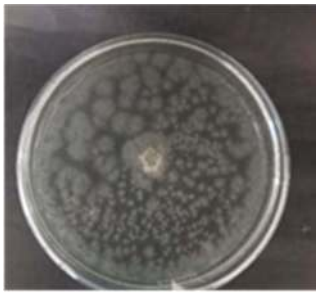


Figure 3. Colonies of the fungus *Aspergillus fumigatus*

Isolates SA2, WA1 and WA5 are morphologically characterized by irregular colony shapes and edges, raised elevations, rough surfaces, light green colonies and pale green colony backs. Microscopically, the parts include conidiophores, vesicles and green conidia. Macroscopically and microscopically this type of fungus is *Aspergillus flavus*. In accordance with what Abbas et al, (2021) stated, that the colonies are initially yellow but quickly turn dark yellow green as the incubation time increases. Conidia are usually radiating and conidiophores are hyaline. Jagat (2021) added that the characteristics of A. *flavus* fungal colonies have a yellowish green color with white colony edges.

Figure 4. colony of *Aspergillus flavus* fungus (Cyrilla, 2018)

Isolates WA8, MA4, SA6 and TA2 are *Penicillium* sp. Macroscopic observations of the isolates show characteristics, namely the shape of round or round *colonies* with flat colony edges, *umbonate* elevations, smooth surfaces, dark green colony tops with white edges and white to yellowish bottoms. Microscopic characteristics are characterized by the presence of conidiophores, vesicles, metulae, phialids and conidia. Straight conidiophores with branched conidia. These characteristics are supported by a statement by Pasaribu (2018),

namely *Penicillium* sp. colonies are dark green and the edges are white. Colonies are coarse-textured, irregularly shaped with button-like elevations. Hyphae morphology is hyaline, adhesive, and branched. Conidia grow from elongated and branched conidiophores. On the conidiophores there are phialids that grow at the ends. Pictures of *Penicilium* sp. fungal colonies can be seen in Figure 5 below:

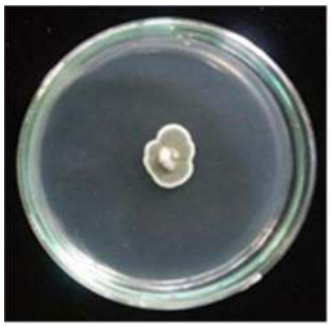


Figure 4. Colony of *Penicillium* sp. fungus (Pasaribu, 2018)

Isolates AA3, AA4, MA1, MA6, SA5, TA1, TA4 and WA4 have morphological characteristics of irregular colony shapes with irregular and diffuse colony edges, some colonies are white to brown in color, the elevation is *raised* with a cotton-like surface. Observation under a microscope shows characteristics such as conidiophores or sporangiophores, round vesicles, and has conidia or sporangium. This is in accordance with the statement of Abbas et al. (2021), namely colonies of *Mucor* sp., grow quickly, white to yellowish or dark gray. Sporangiophores are erect or branched, round in shape, hyaline, gray or brownish in color. The sporangium contains many spores. Pictures of *Mucor* sp. colonies can be seen in Figure 6. below:

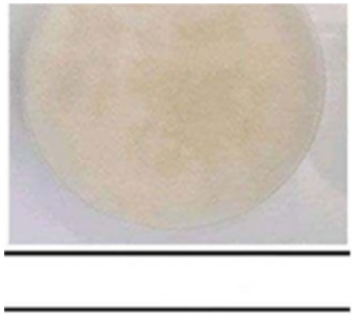


Figure 4. Colonies of *Mucor* sp fungus (Al-Abbasi et al, 2021)

The fungal species *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger* are pathogenic and can cause infections in humans (Nasir, 2017). This infection is called Aspergillosis which causes lung infections in humans (Hasanah, 2017). Penicillium sp. can infect humans and cause diseases that attack human skin, hair and nail tissue called dermatophytosis (Nurhidayah et al., 2021). In addition, one species of *Penicillium fungus*, *Penicillium marneffei*, can cause penicilliosis. Transmission of this infection occurs through

the air and can infect individuals with low immune systems as well as healthy individuals. This infection occurs in the lungs with symptoms of fever, anemia, and weight loss (PERDOSKI, 2017). *Mucor* sp. is one of the fungi from the order *Mucorales* that can cause mucormycosis that infects humans, especially those with immune disorders. This fungal infection is rare but is often found in patients with diabetes mellitus (Mujono *et al*., 2022).

Clean bathroom conditions are needed so that users feel more comfortable when using the bathroom, especially for lecturers and students who use the bathroom in public toilet facilities on campus. In addition, clean toilets can reduce the risk of disease transmission caused by pathogenic fungi. This can be started by routinely cleaning and draining bathroom water in order to suppress the growth of pathogenic fungi in water. Research conducted by Khairani (2020), states that the frequency of draining water can affect the high level of fungal contamination in the water. The longer the draining period, the more mold that contaminates. Conversely, the more often it is drained, the fungal contamination will decrease.

Conclusion

Based on the results of the research that has been done, it can be concluded that the total colonies of fungi isolated from bathroom water samples from 5 different location points are 60 colonies, 47 colonies, 25 colonies, 29 colonies and 46 colonies respectively. The results of isolation of pathogenic fungi in bathroom water samples obtained 31 fungal isolates with a total of 7 fungal species obtained, namely *Aspergillus* sp., *Aspergillus* sp.1, *Aspergillus niger*, A. *fumigatus*, A. *flavus, Penicillium* sp. and *Mucor* sp.

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