

## Characterisation of Non-Dermatophyte Fungus on The Toes of a Patient Diagnosed with Tinea Pedis

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### Abstract

In a health center-level morbidity data report by the Gorontalo City Health Office in 2020, the prevalence of fungal-related skin diseases attained 4.76 percent (476 cases), including 56 cases of tinea pedis. One of the variables in athlete's foot is a workplace and type(s) of work that fail to satisfy basic health guidelines. In the case examined by this study, the patient was a butcher employed locally in a small shop. This study aims to use a variety of parameters to describe the non-dermatophyte fungi that cause butcher's toe infections. Fungus characteristics were performed macroscopically and microscopically. The observations were used to describe the characteristics of the fungus, which were then analysed descriptively with reference to the identification manuals book. Non-dermatophyte fungi such as *Aspergillus* sp., *Rhizopus* sp., *Candida albicans*, were identified in the study findings. *Aspergillus* sp., *Rhizopus* sp., *Candida albicans* and other types of fungi such as *Metarhizium anisopliaea*. This study demonstrates that non-dermatophyta fungal infections may be the source of butchers' tinea pedis.

### Keywords

Butcher's, Non-Dermatophyta Fungus, Tinea Pedis, Toes.

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## INTRODUCTION

There is a considerable variety of irresistible sicknesses in Indonesia, whether caused by fungal pathogens, bacteria, or viruses. A mycotic disease, particularly good superficial mycoses that occur in the nails (onychomycosis) or on

the skin, is one of the diseases that are frequently found in tropical environments such as Indonesia. Personal hygiene and the environment are two of the most important factors in determining whether a disease is caused by a particular pathogen or others (1).

Most skin and nail diseases are caused by dermatophytes, a group of pathogenic fungi. Many dermatophyte fungi have a stratum corneum and keratin (such as skin, scalp, and nails), accordingly, they can easily grow and infect those areas of the human body (2). Additionally, non-dermatophyte companions of the fungal group such as *Candida* spp. and *Aspergillus* spp., are frequently associated with and exacerbated by dermatophyte-caused pathogenic fungal infections (3).

Indonesia is a nation characterised by a climate of high humidity and heat. Thereby making it easier for pathogenic fungal spores that cause superficial mycosis (onychomycosis) to germinate and grow. Pathogenic fungal infections are also increasing, according to recent reports, notably among people with compromised immunity. In addition, Indonesia has a high rate of deep mycosis. The superficial site of deep mycosis attacks the body's surface, infects internal organs, and eventually spreads to the circulatory system (4–6).

As noted by the Gorontalo City Health Office in 2020 in its data report on the level of morbidity at the a local puskesmas, the prevalence of skin diseases caused by fungal infections reached 4.76% (476) cases, one of which was tinea pedis (56 cases). One of the variables causing athlete's foot is a workplace and type(s) of work that fail to satisfy basic work guidelines.

Athlete's foot, also called competitor's foot, is an infection brought about by a contagious dermatophyte disease that contaminates the skin on the toes, bottoms, and parallel pieces of the feet. Athlete's foot represents 20.4% of all dermatomycosis cases. The reason for this illness is the entire lot of dermatophytes, notably *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Athlete's foot infects about 42% of men, compared to 1.7% of women, and its pervasiveness increases with age, although it can also infect children. The signs and side effects of competitor's foot (athlete's foot) are a layered rash that causes chills and irritation (7). Based on previous research, the infection with species from non-dermatophyta groups can also exacerbate dermatocytosis in humans. Therefore, the purpose of this study was to characterise the type of fungi that infects the toes of tinea pedis in butchers.

## MATERIALS AND METHODS

The materials used in this study were Sabouraud Dextrose Agar (SDA) media (14.625 gr), 10% Eosin solution (50 mL), Aquades (225 mL), and 70% alcohol. The samples used were skin scrapings of tinea pedis found on butchers' toes. The samples were extracted from a total of 15 patients.

The research encompassed multiple stages. Initially, the respondents ere prepared for sampling, which involved a systematic

procedure: the skin were cleansing with 70% alcohol. Samples were obtained using a knife, and placed in a plastic clip bag with tweezers. Each sample was meticulously labeled with the corresponding patient's name and age. Subsequently, the samples were prepared for transportation to the Microbiology Laboratory at Bina Mandiri University, Gorontalo, for comprehensive analysis. The subsequent phase involving crafting the growth medium for cultivating the fungi and embedding samples within SDA. The SDA was dissolved in distilled and heated on a hotplate to achieve homogeneity. Following this, the SDA media underwent sterilization in an autoclave at 121°C for 15 minutes (8-10).

The next step was inoculation of the sample on SDA media. It was imperative to utilize gloves and a mask to avoid contamination. The wire loop was heated over a spirit lamp, and a skin scraping was taken in a sterile container using a ring loop. The skin scraping sample was promptly inoculated into specific areas of the SDA media. Subsequently, the petri dish was closed, and the edges were heated over the spirit flame. The media placed in an incubator set at a temperature of 25-28°C for seven days, during which daily observations were conducted. In the event of a mixed culture, the cultivation process was continued to obtain a pure isolate. Each colony on a petri dish was carried out by inoculating a sample of the suspected colony with straight loops, then

inoculating it in the center of sterile SDA media. The media was appropriately labeled with the pertinent details, including the name, date, and sample number. As previously noted, the media was then incubated at room temperature (25-28°C) for seven days. After the incubation period, the fungal culture examination process was initiated.

The third stage involves assessment of growth patterns of the fungal culture daily, both with the naked eye (macroscopic examination) and under a microscope (microscopic examination). The macroscopic analysis encompasses the observation of various aspects, such as colony surface texture (grains, velvet, fine threads like cotton, floccose), colony color, the absence of growth zones, concentration lines or circles, distinctive odor and exudate droplets. Meanwhile, the microscopic examination employs a direct technique. A drop of eosin solution is applied to the object glass, and fungal colonies are carefully collected using a sterile loop, placed on the slide and made like a smear, then covered with a cover glass. The preparations were observed under a 10× or 40× magnification microscope.

This microscopic examination focuses on the structure or arrangement of hyphae and fungal spores (9-20). The final step involves to enforce the results of the inspection. A positive result (+) signifies the presence of on-dermatophyte fungi within the fungal

culture. Conversely, a negative result (-) is assigned when the examination detects non-dermatophyte fungi.

## RESULTS

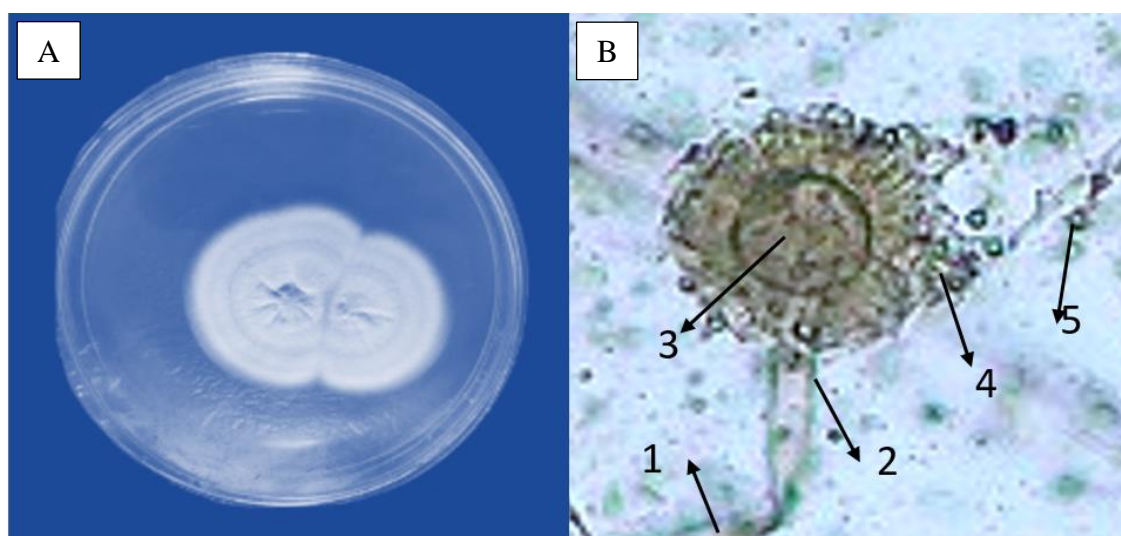
Seven different types of non-dermatophyta fungal isolates were successfully identified from the butcher's feet's skin scrapings, according to the examination's findings. These non-dermatophyte growths included *Aspergillus* sp., *Candida albicans*, *Rhizopus* sp., and various species, such as *Metarhizium anisopliaea*. The results of identifying the four kinds of fungi are depicted in the Figure 1-6 and described in the Table 1-6 for detailed observation.

### Fungus Pure Isolate 1

The macroscopic results of pure fungi isolate 1 are shown in the Figure 1. These results are taken through a magnifying glass

(lup). The characteristics obtained from the macroscopic observations as shown in Figure 1, are presented in detail in Table 1. Similarly, the microscopic result of pure fungus isolates 1 are documented in Figure 1, acquired through a 400x magnification microscope. The characteristics obtained from the microscopic observations of fungus isolates 1 are outlined in Table 1.

Based on the results of macroscopic and microscopic observations of pure fungal isolate 1, it has the characteristics of white colonies with a mixture of yellow inside, the reverse color is yellowish white with smooth colonies. Hyphae are not septate. Conidiophores are colorless (hyaline), smooth and unbranched. Phialide grows on metulae with a round shape with a brownish color. Presence of round brownish vesicles with transparent conidia of rough round shape.



**Figure 1.** Colony morphology of pure fungus isolates 1 on SDA isolated from skin in this study – Isolate top view (A). Microscopic characteristics of pure fungus isolates 1 at 400 x magnification (B) 1. Hyphae; 2. Conidiophores; 3. Vesicles; 4. Phialide; 5. Conidia.

**Table 1.** Results of Macroscopic and Microscopic Characteristics of Pure Fungal Isolates 1

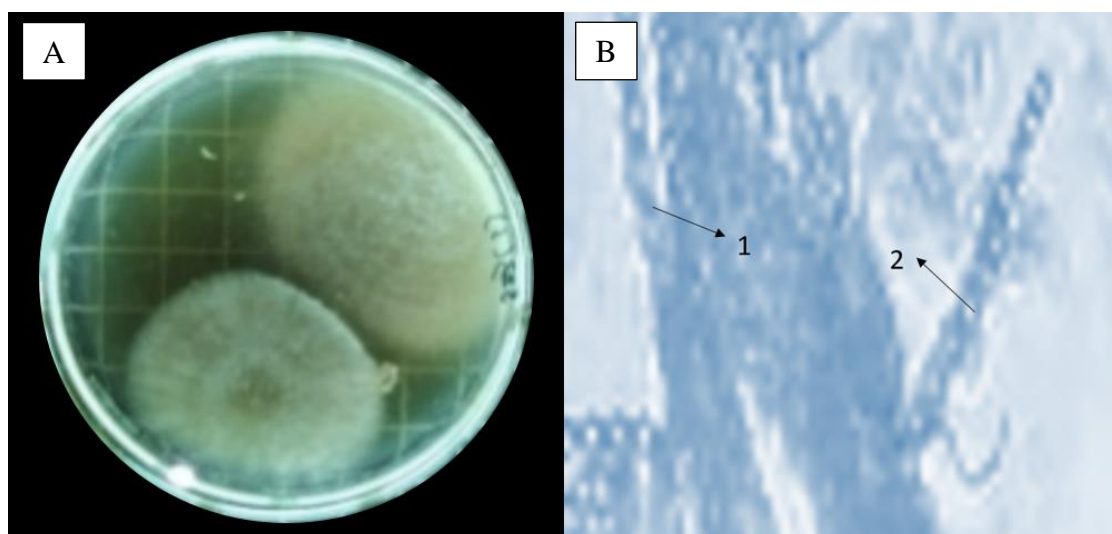
Culture characteristics on SDA	Morphology
<b>Macroscopic</b>	
The color of colony	Yellowish white
Base color on medium	Yellowish white
Reverse sides of the colony	Yellowish white
Properties of the colony	Cotton colonies
<b>Microscopic</b>	
Hyphae form	Non-septate
Conidiophore	Smooth, uncoloured, and unbranched
Vesicle	Round shape and chocolate colour
Phialides	Growing on Metulae, round form and with chocolate colour
Conidia	Round with rough/Transparent

Based on these characteristics, fungal isolate 1 is included in the species *Aspergillus* sp., which can be classified as follows:

Kingdom : Fungi  
 Phylum : Ascomycota  
 Subphylum : Pezizomykotina  
 Class : Eurotiomycetes  
 Order : Eurotiales  
 Family : Trichocomaceae  
 Genus : *Aspergillus*  
 Species : *Aspergillus* sp.

### Fungus Pure Isolate 2

Macroscopic observation of pure fungus isolates 2 obtained the characteristics as shown in Table 2, based on Figure 3 Macroscopically. Based on microscopic observations made on pure fungal isolates 2, the characteristics as shown in Table 2 were obtained, based on microscopic observations in Figure 4.



**Figure 2.** Colony morphology of pure fungus isolates 2 on SDA isolated from skin in this study – Isolate top view (A). Microscopic characteristics of pure fungus isolates 1 at 400 x magnification (B) 1. Conidiophore; 2. Conidia.

**Table 2.** Results of Microscopic Characteristics of Pure Fungal Isolates 2

Culture characteristics on SDA	Morphology
<b>Macroscopic</b>	
The color of colony	Green, yellowish white
Base color on medium	Green, yellowish white
Reverse sides of the colony	Yellowish white
Properties of the colony	Coarse powder
<b>Microscopic</b>	
Conidiophore:	Smooth, uncoloured, and unbranched
Conidia	Round with rough/transparent

The macroscopic and microscopic observations of fungus isolate 2 revealed green colonies that were a mixture of yellowish-white and yellowish-white, with the colonies' shape resembling that of a rather coarse powder. Conidiophores develop upstanding, spores are round and hollow or oval with one cell, conidia are oval in shape. Conidiophores, which are short, branched, close together, and entwined, are formed by some of these branches expanding upward. The following is a classification for fungal isolate 3 based on these characteristics:

Kingdom : Fungi  
 Division : Eumycota  
 Class : Deuteromycetes  
 Order : Moniliales  
 Family : Moniliaceae  
 Genus : *Metarhizium*  
 Species : *Metarhizium anisopliae*

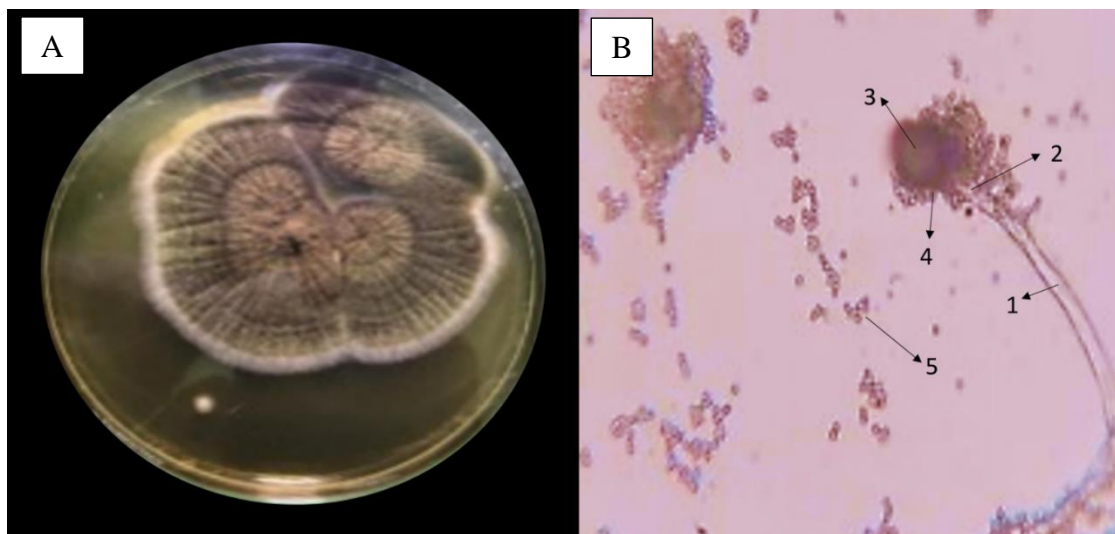
### Fungus Pure Isolate 3

Macroscopic observations of fungus isolate 3 obtained the characteristics as in Table 3, based on the observations in Figure 3. Based on microscopic observations made on fungal isolates 3 (Figure 3), the

characteristics are shown in Table 3. From plainly visible and infinitesimal perceptions (Figure 3), unadulterated fungal confine 3 has the accompanying qualities: black colonies with white margins; smooth colonies in the reverse color are yellowish-white.

Hyphae are not septate. Conidiophores are smooth, unbranched, and colorless (hyaline). The presence of round tanish vesicles with conidia dim brown, harsh round shape. *Aspergillus niger* the surface form of the colony is embossed with a smooth texture on PDA media. *A. niger* has vesicle diameters ranging from 17.52 to 23.4  $\mu\text{m}$ . The conidia are globose with a diameter ranging from 3.5 to 4.5  $\mu\text{m}$  (18). In view of these attributes, fungal detaches 4 can be named follows:

Kingdom : Fungi  
 Phylum : Ascomycota  
 Subphylum : Pezizomykotina  
 Class : Eurotiomycetes  
 Order : Eurotiales  
 Family : Trichocomaceae  
 Genus : *Aspergillus*  
 Species : *Aspergillus niger*



**Figure 3.** Colony morphology of pure fungus isolates 3 on SDA isolated from skin in this study – Isolate top view (A). Microscopic characteristics of pure fungal isolates 3 at 400 x magnification. 1. Hyphae; 2. Conidiophores; 3. Vesicles; 4. Phialide, 5. Conidia.

**Table 3.** Results of Macroscopic Characteristics of Fungal Isolate 3

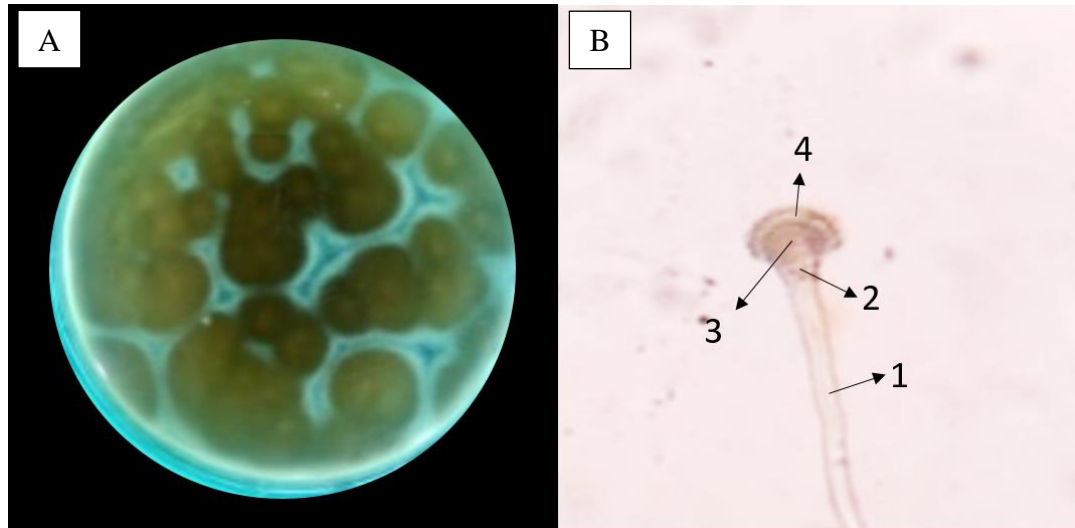
Culture characteristics on SDA	Morphology
<b>Macroscopic</b>	
The color of colony	Black
Base color on medium	Black
Reverse sides of the colony	Yellowish white
Properties of the colony	Fine
<b>Microscopic</b>	
Hyphae form	Non-septate
Conidiophore:	Hyaline, smooth, and unbranched
Vesicle	Round shape and chocolate colour
Phialides	Growing on Metulae, round form and with chocolate colour
Conidia	Round with rough/dark chocolate colour

### Fungus Pure Isolate 4

The pure isolates obtained were identified and observed macroscopically and microscopically. Macroscopic observation of pure fungal isolates 4 showed the characteristics shown in Figure 4. Based on microscopic observations made on fungal isolates 4, the characteristics as shown in Table 44 were obtained. For more details, microscopic observations on the isolation media can be seen in the Figure 4.

From macroscopic and microscopic observations (Figure 4), the isolates 4 fungus has the characteristics of a bright green slightly brown colony, the reverse color is yellowish white with smooth colonies. Hyphae are not septate. Conidiophores are colorless (hyaline), smooth and unbranched. The presence of round brownish vesicles with conidia dark brown, rough round shape (21). Based on these characteristics, fungal isolates 4 can be classified as follows:

Kingdom	: Fungi	Order	: Eurotiales
Phylum	: Ascomycota	Family	: Trichocomaceae
Subphylum	: Pezizomykotina	Genus	: <i>Aspergillus</i>
Class	: Eurotiomycetes	Species	: <i>Aspergillus terreus</i>



**Figure 4.** (A) Colony morphology of pure fungus isolates 4 on SDA isolated from skin in this study – Isolate top view. Microscopic characteristics of pure fungal isolates 4 at 400 x magnification. (B) 1. Hyphae; 2. Conidiophores; 3. Vesicles; 4. Conidia

**Table 4.** Results of Macroscopic Characteristics of Fungal Isolate 4

Culture characteristics on SDA	Morphology
<b>Macroscopic</b>	
The color of colony	Brownish Green
Base color on medium	Brownish Green
Reverse sides of the colony	Yellowish White
Properties of the colony	Fine cotton
<b>Microscopic</b>	
Hyphae form	Non-septate
Conidiophore	Hyaline, unbranched, and smooth branching
Vesicle	Round shape and chocolate colour
Phialides	Growing on Metulae, round form and with chocolate colour
Conidia	Round with rough/dark chocolate colour

### Fungus Pure Isolate 5

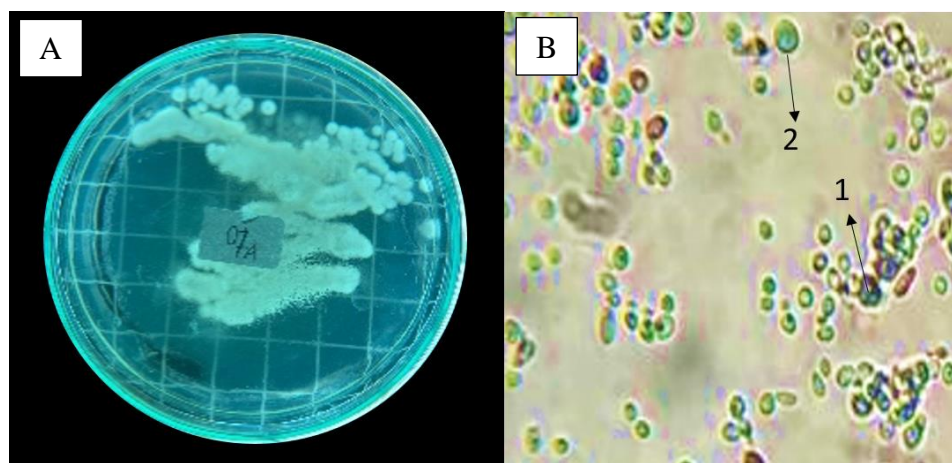
Macroscopic observation of pure fungus isolates 5 obtained the characteristics as shown in Table 5, based on Figure 5. Based on microscopic observations made on fungal isolates 5, the characteristics shown in Table 5 were obtained, as shown in Figure 5.

Macroscopic and microscopic observations of fungus isolates 5 had characteristics, namely round, oval, oval shape. Blastospores and Chlamydo-spore are round or oval in shape. The surface of the colony is smooth, smooth, slightly convex, slightly wet, shiny round, and yellowish-



white in color with a yellow reverse color. These characteristics are the macroscopic characteristics of the *Candida albicans* fungus, which has a sour smell, has yeast-like colonies, is white in color and has a convex surface (10). Based on these characteristics, fungus isolates 5 can be classified as follows:

Kingdom : Fungi  
 Phylum : Ascomycota  
 Subphylum : Saccharomycotina  
 Class : Saccharomycetes  
 Order : Saccharomycetales  
 Family : Saccharomycetaceae  
 Genus : *Candida*  
 Species : *Candida albicans*



**Figure 5.** (A) Colony morphology of pure fungus isolates 5 on SDA isolated from skin in this study – Top view isolate. (B) Microscopic characteristics of pure fungal isolate 5 at 400 x magnification. 1. Blastospore; 2. Chlamydospore

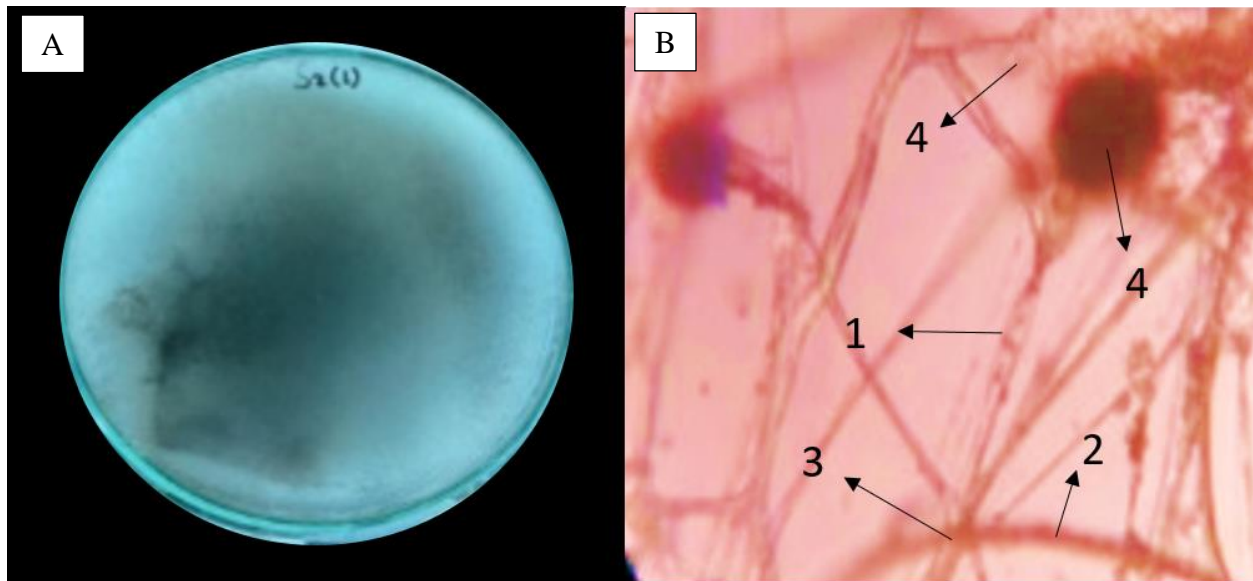
**Table 5.** Results of Macroscopic Characteristics of Fungal Isolate 5

Culture characteristics on SDA	Colony morphology
<b>Macroscopic</b>	
The color of colony	Yellowish white
Base color on medium	Yellowish white
Reverse sides of the colony	Yellow
Properties of the colony	Round
<b>Microscopic</b>	
Blastospore	Round and oval shape with uncoloured
Chlamydospore	Round and oval shape with uncoloured

### Fungus Pure Isolate 6

The pure isolates obtained were identified and observed macroscopically and microscopically. From macroscopic observations of fungus isolates 6, the

characteristics shown in Table 6 were obtained, according to those in Figure 6. Based on microscopic observations made on fungus isolates 6, the characteristics shown in Table 6 were obtained, according to Figure 6.



**Figure 6.** (A) Colony morphology of pure fungus isolates 6 on SDA isolated from skin in this study – Top View Isolate. (B) Microscopic characteristics of pure fungus isolates 6 at 400 x magnification 1. Hyphae; 2. Stolons; 3. Rhizoids; 4. Sporangifora; 5. Sporangium

**Table 6** Results of Macroscopic Characteristics of Fungal Isolate 6

Culture characteristics on SDA	Morphology
<b>Macroscopic</b>	
The color of colony	White gray
Base color on medium	White gray
Reverse sides of the colony	Brownish white
Properties of the colony	Cotton
<b>Microscopic</b>	
Hyphae form	Non-septate
Stolons	Characteristic smooth with chocolate colour
Rhizoids	Branched with chocolate colour
Sporangiophore	Chocolate colour
Sporangium	Round shape with fine/Dark chocolate colour

Macroscopic and microscopic observations of the pure isolate of fungus 6 reveal that it exhibits characteristics of white to gray colonies, with a brownish-white reverse color. The colony structure appears cottony in nature. It possesses hyphae that form rhizoids to attach to the substrate, and has coenocytic hyphae that lack septa or have septa. Stolons spread over the substrate, accompanied by sporangiophores with upwards-growing stalks containing numerous spores, as well as large, dark

brown, smooth-walled sporangia (20). Based on these characteristics, fungus isolate 6 belongs to the *Rhizopus* sp. species, which can be classified as follows:

Kingdom	: Fungi
Division	: Zygomycota
Class	: Mucoromycotina
Order	: Mucorales
Family	: Mucoraceae
Genus	: <i>Rhizopus</i>
Species	: <i>Rhizopus</i> sp.

## DISCUSSION

In view of the results of observing tests of skin scrapings from meat dealers in small butchers' shops in Gorontalo city, which were undertaken utilising the way of life strategy on Saboraud Dextrose Agar (SDA) media, non-dermatophyte parasites were found, notably *Aspergillus* sp, *Aspergillus niger*, *Aspergillus terreus*, *Rhizopus* sp, *Candida albicans* and different species, for example, *Metarhizium anisopliaea*.

Fifteen examples of foot skin scrapings which were analysed visibly and minutely from meat vendors in customary business sectors in Gorontalo City, subsequent to being consulted 12 respondents were found to not constantly wear shoes or other foot coverings when moving about and selling meat. Of these 12, three only occasionally wore shoes. The average type of fungus obtained from 12 respondents determined that the most isolates detected were the fungus *Aspergillus* sp. (13).

Microscopically, the fungus *Aspergillus* sp. has hyphae that branch and are partitioned, while the conidiophores do not have branching but some have partitions and do not have partitions depending on the type of species. Free-living and widespread contaminant fungi include *Aspergillus* sp., *Aspergillus niger*, and *Aspergillus terreus* (14). The air is where the fungus *Aspergillus* sp. can spread, and it can contaminate in unfavorable conditions such as a humid

environment. *Aspergillus* sp. is hazardous as it can cause contaminations in the human body such as sensitivities, and in addition it can cause diseases in the human lungs (13).

The way of life aftereffects of skin scratching tests additionally found growths of the family *Rhizopus* in samples of foot skin scrapings. One of the pathogenic fungi that can infect the human body and cause inflammation or infection is the fungus *Rhizopus* sp. Humidity has an impact on the likelihood of detecting the fungus *Rhizopus* sp. in samples. For fungi to grow, humidity is all-important. For example, the fungus *Rhizopus* sp. needs a low-level environment with 90% humidity. As well, the *Rhizopus* sp. In small skin wounds, dermatophytosis fungi can also spread by direct contact (15).

Furthermore, analysts additionally found *Candida albicans* organism. This is a type of fungus that infects the skin and thrives in warm, humid environments (16). The chance of the development of the *Candida albicans* parasite can occur when there are inclining variables, for example, neatness of the skin of the feet, the propensity for absorbing the feet water for a long time, causing maceration which makes it easier for the development of the *Candida albicans* organism, and warm and moist environments cause expanded sweat. *Candida albicans* is considered as the most pathogenic species and the most common cause of candidiasis, a fungal disease that attacks the skin, hair, nails,

mucous membranes and internal organs (10). The species *C. albicans* is among the most important and most frequently isolated from healthy and diseased persons and is considered a commensal organism and, at the same time, a pathogen causing health complications. *C. albicans* represents over 80% of isolates from all forms of human candidosis (22), (23). This makes it workable for the parasite *Candida albicans* to be present in the foot skin scratching test.

The fungus *Metarhizium anisopliae* was also discovered in this study. *Metarhizium anisopliae* is one of the endopathogenic parasites that has the potential for bother control, which can lessen bug populaces in a rural region contrasted with different organic entities. It is conceivable that the organism *Metarhizium anisopliae* was found in the butcher's foot scratching test on the grounds that at the time preceding examining, the respondent was taking creature feed from the ranch region. The growth *Metarhizium anisopliae* ordinarily occurs in plants like corn, rice, sugar cane and is typically found in soil. The fungus *Metarhizium anisopliae* is parasitic and saprophytic in the soil by surviving on plant debris. *Metarhizium anisopliae* is widely used to treat insect larvae and plant-disturbing caterpillars (17).

Some of the respondents complained of tingling between the toes and also of moistness. As per Nigam and Saleh (24), the development of Athlete's foot on the feet is

brought about by the state of the feet which are wet and hot when wearing foot wear. This can cause the development of Non dermatophyta growths, and taint between the butcher's toes, causing athlete's foot.

## CONCLUSIONS

Based on the findings from macroscopic and microscopic examinations conducted among meat sellers with tinea pedis patient in Gorontalo City, various groups of non-dermatophyte fungi were identified. These groups included species such as *Aspergillus* sp., *Aspergillus niger*, *Aspergillus terreus*, *Rhizopus* sp., *Candida albicans*, as well as others like *Metarhizium anisopliae*.

## AUTHOR CONTRIBUTIONS

Yolan Dunggjo: Planning and designing research, processing research results, interpreting results, and compiling research manuscripts. Ismail Mukusibu: Took samples at the location, prepared tools and materials, and looked for references related to the research.

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Bina Mandiri University.

## CONFLICT OF INTEREST

There are no conflicts of interest.

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