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Microbial exploration from two different ecosystems in Central Sulawesi, Indonesia

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Abstract. *Ratnawati, Sudewi S, Jaya K, Saleh AR. 2022. Microbial exploration from two different ecosystems in Central Sulawesi, Indonesia. Biodiversitas 23: 6089-6096.* A supportive growing environment and the presence of microbes in the soil are considered good habitats for plant growth. Endophytic microbes live in symbiosis (mutual benefit) with their host plants, both in the stem and root tissues of plants, providing defense services in biotic and abiotic stress situations. This study was aimed to identify various microbes that exist in various agricultural cultivation ecosystems in Central Sulawesi. Microbial sampling was obtained from healthy plant tissues (endophytes) and rhizosphere in lowland rice and shallot plantation ecosystems. The sample obtained was then weighed as much as 5 g and surface sterilized for further isolation by serial dilution method to be spread on NA (*Nutrient Agar*) media for bacteria, and PDA (*Potato Dextrose Agar*) for fungi. The microbes that had grown were re-isolated to obtain pure isolate culture of microbes. The results showed that 8 isolates from the rhizosphere and 4 isolates as endophytes in the ecosystem of organic rice fields were recorded. The fungal isolates from the shallot plantation "Lembah Palu" found as many as 12 isolates from rhizosphere and 4 isolates as endophytes. The results of macroscopic and microscopic characteristics on fungal isolates identified as the species of *Trichoderma, Fusarium, Penicillium, Gliocladium* and *Aspergillus.*

Keywords: Endophytes, microbes, paddy, rhizosphere, shallot

INTRODUCTION

Indonesia is a tropical country rich in biodiversity in the form of microbial biodiversity. The presence of microbes is very important in agroecosystems in order to maintain the balance of the soil ecosystem, nutrient cycles and plant productivity (Mendes et al. 2017). In general, microbial diversity includes various types of microorganisms such as bacteria, fungi, viruses and nematodes (Onen et al. 2020).

Central Sulawesi is one of the provinces that has a specific tropical climate because it is an area that is crossed by the equator. This area allows storing rich sources of microbial biodiversity in various existing ecosystems. Tropical ecosystems are the main habitat for a rich diversity of microbial populations compared to temperate ecosystems (Pajares et al. 2016). Biodiversity in various ecosystems that could be found in Central Sulawesi Province include forests, rice fields, gardens and grasslands. Microbes from various ecosystems are very important for life (Cavicchioli et al. 2019), especially for plants because they have the function and potential to be utilized for plant growth and productivity (Harman et al. 2021; Dobrovol'skaya et al. 2015).

The interaction between microbes and plants is an indicator of soil fertility (Saleh et al. 2022). Use of beneficial microbes help in improving soil texture and structure by increasing its aggregation and stability (Rashid et al. 2016). Their in agricultural cultivation has become a trend for many researchers, especially in relation to plant growth boosters that play a role in dissolving potassium and phosphate (Jaya et al. 2021; Siddiqui et al. 2021; Gupta et al. 2021; Sherpa et al. 2021), production of phytohormones and siderophores (Sudewi et al. 2021; Tamreihao et al. 2022; Maulina et al. 2022; Nguyen et al. 2022) and fixation of nitrogen (Haerani et al. 2021; Zhang et al. 2022; Khatun et al. 2021; Setiawati et al. 2022). Microbes can also increase plant resistance to pests and diseases by producing antagonistic compounds (Al-Ani et al. 2020). Marwan et al. (2021) reported that isolates of endophytic bacteria from local rice were able to suppress the growth of *Pyricularia oryzae* causing blast disease by 26.56–79.69%, *Beauveria bassiana* Bals could control important pests of rice (Sopialena et al. 2021), green ladybug (Siahaan et al. 2021), insect pest (Ramakuwela et al. 2020). Biological control of fungal diseases by *Trichoderma* sp. can suppress the development of moler disease (Sánchez-Montesinos et al. 2021; Mulyana et al. 2021) and tuber rot on onion plants (Sudantha et al. 2020; Soesanto et al. 2018).

Plant rhizosphere is an ideal habitat for the growth and development of microbes (bacteria and fungi) (Xiong et al. 2021). The abundance and diversity of microbial populations in the rhizosphere is strongly supported by nutrients and environmental factors. The rhizosphere is very rich in nutrients in the form of amino acids and sugars that are secreted into plants (Liu et al. 2022). In addition, the plant tissue is also a suitable habitat for various types of bacteria and fungi. Different habitats will provide different

microbial population diversity (White et al. 2019; Putrie et al. 2020). Exploration results (Ratnawati and Jaya 2021) revealed different types of endophytic fungi were found associated namely *Penicillium* sp., *Trichoderma mf1*, *Aspergillus niger*, *Fusarium* sp., *Trichoderma mf2*, *Aspergillus flavus*, *Trichoderma mf3*, *Fusarium* sp, *Trichoderma mf4* with organic shallot plantations. The abundance of existing microorganisms encourages the isolation of plant parts from various food cropping and horticultural ecosystems in Central Sulawesi.

Rice and shallots from the "Lembah Palu" are two of Central Sulawesi's main commodities that receive priority to be developed in a sustainable manner. Land conditions that support the development of agricultural commodities also support microbial activity in the vicinity. However, the microorganisms from the Central Sulawesi "Lembah Palu" Rice and Shallots that have not been explored fully for biological microbial diversity so that it becomes interesting to be inventoried by type, developed and used optimally to improve the quality of life, the environment to support the concept sustainable agriculture.

MATERIALS AND METHODS

Study area

Microbial sampling was carried out in the ecosystem of rice fields and organic shallots. The source of bacterial isolates was obtained from the lowland rice ecosystem in Lelio and Bakekau villages of West Lore Sub-district, Poso District, Central Sulawesi, Indonesia. Lelio villages are at the coordinates of 1°54'28.62"S;120°16'32.56"E and Bakekau village 1°54'34.01"S;120°15'27.18"E (Figure 1) West Lore Sub-district has an area of 428.2 km^2 with an altitude of ± 1000 m asl. While fungal isolates were obtained from the "Lembah Palu" shallot plantation ecosystem in Maku Village, Sigi District, Central Sulawesi at the coordinates 1°2'4.81"S119°53'15.97"E. Sampling of endophytic microbes was carried out randomly using purposive random sampling method on healthy rice and shallot plants with morphological characteristics, namely the growth of plants was better than others in a population of rice fields and onion plantations. Rhizosphere microbial samples were obtained by taking samples of soil and plant roots with a depth of 1-10 cm in the ecosystem of rice fields and onion plantations. The method used is diagonal sampling by determining 10 different sampling points. Each sample was collected and put in a sterile brown envelope, labeled and stored in a cooler box, then brought to the laboratory for isolation and further testing before 48 hours after collection from the location (Borkar 2018).

Procedures

Microbial isolation

Isolation of endophytic microbes (bacteria and fungi) was carried out by washing samples of rice and shallot roots under running water to remove other particles adhering to the roots. Furthermore, the roots were dried with tissue paper and then cut into small pieces with a size of 1-2 cm. The samples of rice roots and shallots were surface sterilized according to the modified Hallmann et al. (1997) method. Surface sterilization was carried out by soaking each root of the plant in 70% alcohol for 1 minute, then soaking it in 2.5% sodium hypochlorite (NaOCl) for 2 minutes, then rinsing with sterile water three times and drying on the surface of the cup, covered with sterile tissue.

The effectiveness of the results of surface sterilization was tested by placing pieces of rice roots on NA medium and pieces of onion roots on PDA media and incubated for 48 hours at room temperature.

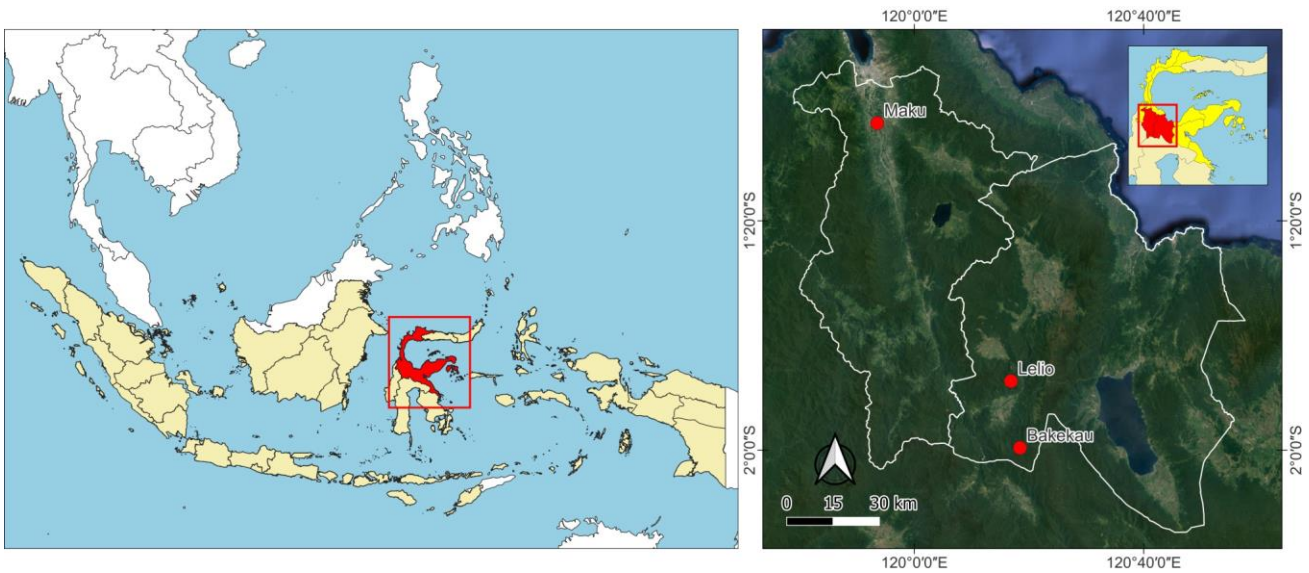

Figure 1. Locations of sampling sites in Poso and Sigi District, Central Sulawesi, Indonesia. The Map is modified from Google Maps Platform

The indicator of the success of sterilization is by observing the roots that are not overgrown by contaminant microorganisms in each of these media so that they can be used as isolation materials. A total of 5 g of roots were weighed and crushed using a sterile mortar until smooth, then 1 ml of root suspension was taken and mixed with 9 ml of sterile water in a test tube. The root suspension was then diluted using the serial dilution method with concentrations of 10^{-2}, 10^{-3}, and 10^{-4}. A total of 0.1 ml of each dilution was spread on NA medium for bacteria and PDA medium for fungi.

Isolation of rhizosphere microbes was carried out with reference to the method of Sharma and Shrivastava (2017), which modified composites of rhizosphere soil samples weighing 10 g each and then mashed using a sterile mortar and then diluted to a concentration of up to 10^{-4}. The soil sample suspension that has been diluted is then taken as much as 0.1 ml to be spread on NA and PDA medium. Furthermore, each sample (endophytic and rhizosphere microbes) was incubated at room temperature (28±2ºC) for 3-7 days to obtain bacterial and fungal isolates. The growing single colonies were re-isolated in each medium (NA and PDA) in order to obtain pure cultures of microbial isolates. Purification was carried out on each bacterial and fungal colonies, which were considered different based on their morphology. If the morphological characteristics show the same shape and color, it is considered one type. For further testing, microbial isolates with an incubation age of 2-3 days were used.

Characterization of morphology and physiology of bacterial isolates

Single colonies from pure cultures of bacterial isolates were characterized by macroscopic morphology by visually observing colony size, pigmentation (colony color), colony shape, colony edge and elevation. Physiological characteristics were carried out by Gram reaction test and catalase enzyme production test. Observations of the Gram reaction test by taking 1 loop full of bacterial isolates and placing it on a glass object that had previously been dripped with 3% KOH (*Potassium Hydroxide*) solution. The indicator for the Gram reaction is positive if the bacterial isolate looks sticky like thread and slimy when it is lifted from the surface of the suspension on a glass object, and vice versa (Suslow et al. 1982). The catalase test was carried out by adding 1 full ose of pure bacterial single colony culture, then smeared on a slide that had been given two drops of 3% H_2O_2 (*Hydrogen Peroxide*). The emergence of gas bubbles from free oxygen is an indicator of a positive reaction, while a negative reaction does not indicate the presence of gas bubbles.

Macroscopic and microscopic identification of fungal isolates

Identification of fungal isolates was carried out macroscopically and microscopically. Pure cultures of fungal isolates were observed visually with macroscopic characteristics based on the color and surface of the colonies, the color of the hyphae, referring to the method proposed by Salvamani and Nawawi (2014), while microscopic observations were carried out by looking at the shape of the conidia, using a digital microscope. Aseptically pure cultures of fungal isolates were taken using a needle and then placed on a slide, then dripped with lactophenol cotton blue dye, then covered with a slide and observed under a microscope with first 10X, then 40X and 100X magnification. The characteristics observed microscopically include the structure of hyphae and reproductive structures. Microscopic observations of fungal isolates were compared with identification books by Barnett and Hunter (1998).

RESULTS AND DISCUSSION

Initial isolation of endophytic and rhizosphere microbes obtained from the lowland rice and shallot plantation ecosystems "Lembah Palu" showed that there were 38 bacterial isolates and 44 fungal isolates found. However, after the next selection, there were microbial isolates whose colony growth was 50%, some had the same character when observed macroscopically, so that at the final stage of selection, 8 bacterial isolates from the rhizosphere and 4 isolates from endophytes in the ecosystem of organic rice fields and 12 fungal isolates from rhizosphere and 4 isolates from endophytes in shallot plantation ecosystems.

Characterization of bacterial isolates

The results of the morphological characterization of bacterial isolates found various colony size characteristics, namely 3 large isolates, 4 moderate isolates, 3 small isolates and 2 punctiform isolates. The colony shape was dominated by circular shape with 9 isolates, the remaining 3 isolates irregular. The edges of the bacterial colonies obtained were undulate 7 isolates, total 4 isolates and curled 1 isolate. The elevation was dominated by flat as many as 7 isolates, 3 isolates with convex elevation and 2 isolates with raised elevation. The colony colors obtained were 5 isolates yellow, 5 isolates cream color, 2 isolates white (Table 1; Figure 2). The color of the colonies of different isolates was due to the presence of different pigments in the constituent cells of the bacterial isolates (Herwati et al. 2021; Rao et al. 2017; Venil et al. 2020). Overall observations of the morphological characteristics of bacterial isolates showed different results. This is because the bacterial isolates found came from the rice field ecosystem with different environmental conditions and the source of the bacterial isolates came from the endophytic and rhizosphere of rice plants.

Physiological characterization of bacterial isolates was carried out by gram reaction test and catalase reaction. The results of the Gram test showed that 7 isolates were classified as Gram-positive (+) and 5 isolates were classified as Gram-negative (-) (Table 1). Gram-positive bacteria have thick and rigid cell walls because they are composed of peptidoglycan with more composition than Gram-negative bacteria have thin cell walls, so the Potassium Hydroxide used in the test will more easily break down the cell walls of Gram-negative bacteria. (Wang et al. 2020; Sudewi et al. 2020). The catalase test on bacterial isolates found that 15 isolates reacted positively

and one isolate reacted negatively. The isolates of catalase positive bacteria were able to break down the hydrogen peroxide used in the test into oxygen and water while the catalase negative bacteria were unable (Babiker et al. 2016; Yuan et al. 2021).

Characterization of fungal isolates

Macroscopic observation of the fungal isolates from the shallot plantation "Lembah Palu" (Table 2) found as many as 16 isolates that had different morphological characterizations based on colony development in terms of colony color (top view and bottom view), colony shape and hyphae. Microscopic identification results obtained 2 fungal isolates (UR1, UR2) which were not identified correctly. This was because the isolates were in the form of conidia/spores and the hyphae were not clearly visible, so further identification was necessary for this isolate. A total of 14 isolates were identified belong to species of *Trichoderma*, *Fusarium*, *Penicillium*, *Gliocladium* and *Aspergillus.*

Table 1. Characteristics of colony morphology and physiology of bacterial isolates from rice cultivation

Isolate code	Size	Colony form	Colony edge	Elevation	Colony color	Gram (+/-)	Catalase (+/-)
DW01	Small	Circular	Undulate	Flat	Yellow	(-)	(+)
DW02	Large	Circular	Entire	Raised	Cream	(+)	(+)
DW03	Large	Irregular	Undulate	Flat	White	(+)	(-)
DW04	Large	Irregular	Curled	Flat	Cream	(+)	(+)
DW05	Moderate	Circular	Undulate	Convex	White	(+)	(+)
DW06	Moderate	Circular	Entire	Flat	Cream	(+)	(+)
DW07	Punctiform	Circular	Undulate	Convex	Cream	(+)	(+)
DW08	Small	Circular	Undulate	Flat	Yellow	(-)	(+)
DW09	Moderate	Irregular	Undulate	Raised	Yellow	(-)	(+)
DW10	Small	Circular	Undulate	Flat	Cream	(-)	(+)
DW11	Moderate	Circular	Entire	Flat	Yellow	(+)	(+)
DW12	Punctiform	Circular	Entire	Convex	Yellow	(-)	(+)

Note (+) : positif reaction (-) : negatif reaction. Isolate code DW01-DW08 from the rhizosphere of rice plants, DW09-DW12 from endophytic sample.

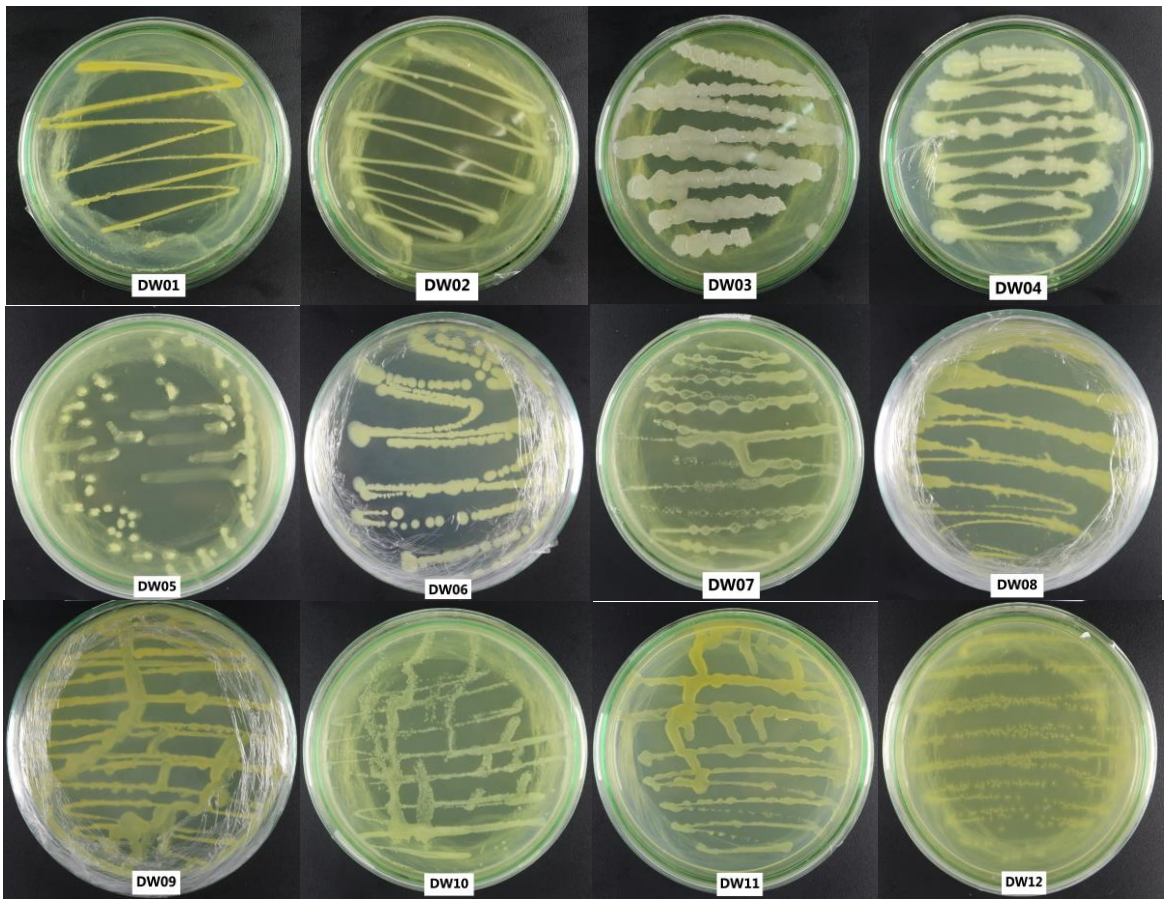

Figure 2. Pure culture of isolate bacteria from rice cultivation

The results of macroscopic and macroscopic observations on isolates TR1, TR2, TR3, TR4, TR5 were identified as the genus *Trichoderma* because the isolates of this fungus have characteristics that are in accordance with *Trichoderma* sp. Isolates TR 1 to TR5 had the same hyphal color and conidia shape, namely hyaline hyphae and round conidia. The difference lies in the color of the colonies on the upper and lower surfaces (Table 2). Isolate TR1 (top view) had a white colony color then the mycelium turned green in the middle, with a pale green halo border, a thin and rough cotton-like texture, while the color of the colonies observed at the bottom was white. TR2 isolates showed white colonies turning green in the center forming a circle, with pale white edges, thin and coarse cotton-like texture, cloudy white underside.

TR3 isolate with a yellowish-white bottom color, while the top view was white which changed to dark green, forming a repeating circle with a rough texture. The white color looks cloudy white with the top view turning white to dark green forming a circle resembling a ring with a halo in the middle, thick and rough texture (TR4 isolate), while the TR 5 isolate has a white top visible colony color turning green in the middle, with the edges are pale white, forming a ring like a circle, the texture is thick and rough, while the bottom is cloudy white.

The microscopic characteristics of *Trichoderma* sp. (Figure 3) can be seen as conidiophores with many regular branches. If in large quantities, they form conidia groups that are oval in shape and dark green in color which can be seen in the shoot segments. This genus is easily recognized quickly just by visually observing the greenish color of the colony growth. *Trichoderma* sp. is classified as an antagonistic fungus which is found in cultivated land, forest ecosystems, as well as on all types of soil with different ecosystems and categorized based on their metabolic, physiological and genetic diversity features (Hu et al. 2020; Ma et al. 2020; Mulatu et al. 2022).

Discussion

The variable colony color, size, shape, elevation, and colony edges that vary are shown in Figure 2, which is because of the different types of bacteria found. These differences are still based on observations of their morphological characters, not being able to determine the genus or species of each of these bacteria. To determine the type of bacteria, it is necessary to carry out physiological characterization and further molecular identification.

Based on the origin of the isolates obtained from the endophytic tissue and rhizosphere of healthy rice plants, it was found that when cultured the population of bacterial isolates from the rhizosphere was higher than that of the bacterial isolates from plant endophytes. The morphological identification data were 8 bacterial isolates from the rhizosphere and 4 isolates from endophytes. The total before selection was 38 isolates (25 rhizosphere isolates and 13 endophytic isolates). This is in line with the research results (Abedinzadeh et al. 2019; Afzal et al. 2019) that generally the population of endophytic bacteria will be less than that of rhizosphere bacteria and pathogenic bacteria.

Table 2. Macroscopic and microscopic characteristics of colonies and identification of fungal isolate genus from shallots

Isolate code	Colony Color		Hifa		Conidia form	Genus
	Top view	Bottom view	Color	Insulated		
TR1	White, middle green	White	Hialin	Insulated	Round	*Trichoderma* sp. *mf1*
TR2	White, middle green	Cloudy white	Hialin	Insulated	Round	*Trichoderma* sp. *mf2*
TR3	White, middle green	Yellowish white	Hialin	Insulated	Round	*Trichoderma* sp. *mf3*
TR4	White, dark green	Cloudy white	Hialin	Insulated	Round	*Trichoderma* sp. *mf4*
TR5	White, dark green	Cloudy white	Hialin	Insulated	Round	*Trichoderma* sp. *mf5*
FR1	White, thin cotton texture	Yellowish white	Hialin	Insulated	Crescent moon	*Fusarium* sp. *mf1*
FR2	White, thick cotton texture	White	Hialin	Insulated	Crescent moon	*Fusarium* sp. *mf2*
FR3	White, fine cotton texture	Slightly orange white	Hialin	Insulated	Crescent moon	*Fusarium* sp. *mf3*
PR1	Light green with white edges	Cream	Hialin	Insulated	Small round	*Penicillium* sp. *mf1*
PR2	Light green with white edges	Cloudy white	Hialin	Insulated	Small round	*Penicillium* sp. *mf2*
PR3	Light green with white edges	Gray	Hialin	Insulated	Small round	*Penicillium* sp. *mf3*
UR1	White, fine cotton texture	White	Hialin	-	-	*Unidentified mf*1
UR2	White, thick cotton texture	White	Hialin	-	-	*Unidentified mf*2
GR1	Green, dark brown	Cloudy white	Hialin	Insulated	Scattered small round	*Gliocladium* sp. *mf1*
GR2	Green, dark brown	Cloudy white	Hialin	Insulated	Scattered small round	*Gliocladium* sp. *mf2*
AR	Black, white border	Cloudy white	Hialin	Insulated	Small round	*Aspergillus* sp.

Note : Isolate code TR1-TR5, PR1-PR3, UR1-UR2 and GR1-GR2 from the rhizosphere of shallot plants, FR1-FR3 and isolate code AR from endophytic sample.

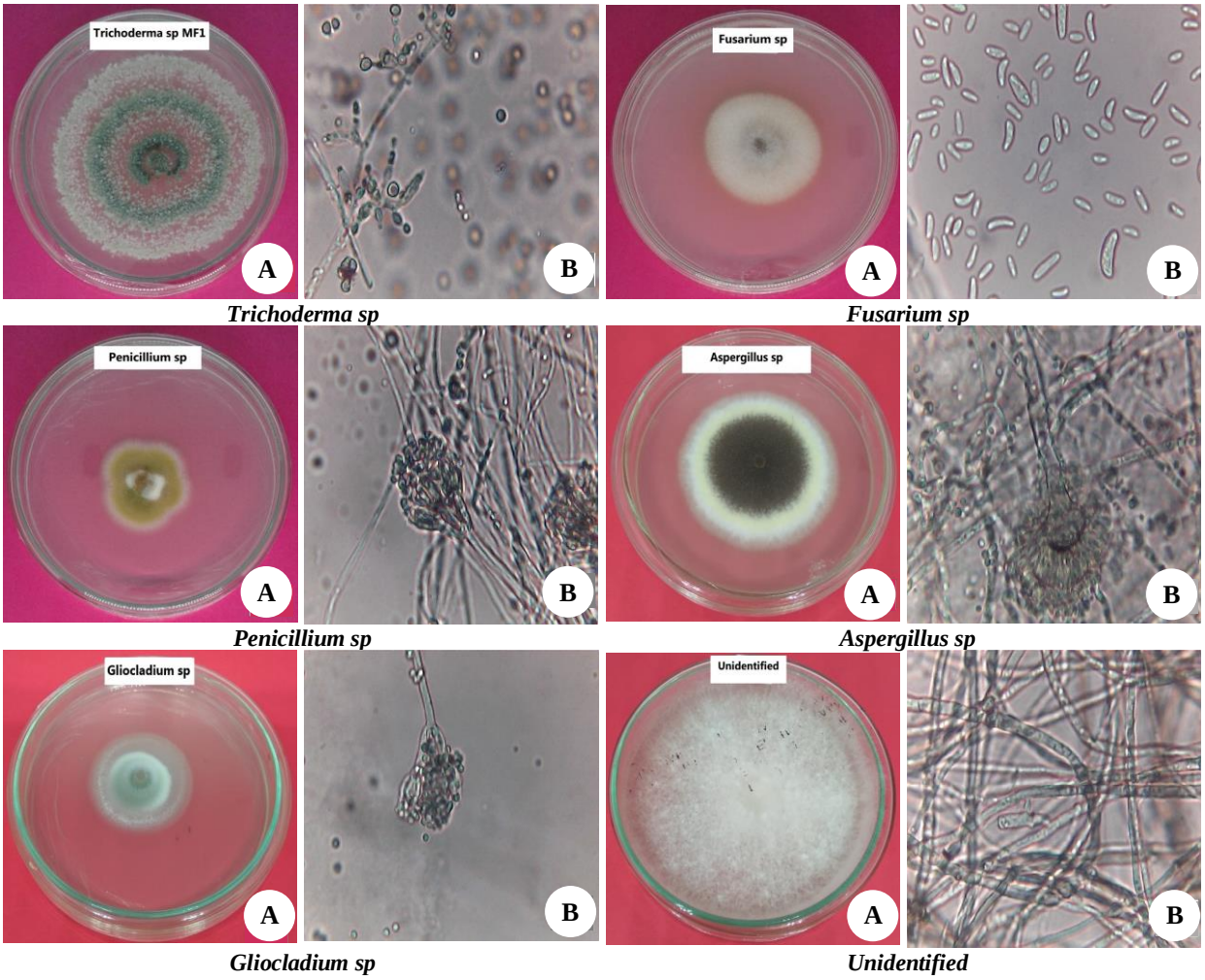

Figure 3. Identified fungal by microscopic characteristics: A. Mycellium growth, B. Microscopic appearence with 100 X magnification

The total before selection was 44 fungal isolates, of which 34 were from rhizosphere and 10 as endophytic isolates. Based the Table 2, isolate code TR1-TR5, PR1-PR3, UR1-UR2 and GR1-GR2 from the rhizosphere of shallot plants, FR1-FR3 and isolate code AR from endophytic sample. Isolates FR1, FR2, and FR3 were macroscopically and microscopically identified in the genus *Fusarium* sp. because they had characters that matched the genus group. These three isolates had white colonies visible from the upper surface with a texture like thin to thick cotton. The underside is yellowish white, white and slightly orange white, hyphae are insulated, hyaline and branched and have conidia shape like a crescent moon with slightly bent ends. In line with research results (Palacio-Barrera et al. 2019; Kalra et al. 2020) that isolates that form sporodocium in large numbers, the color of the colonies will change from white to orange, besides that it is also influenced by the pigment produced, medium used and abiotic with biotic factor. *Fusarium* sp. is classified as an unstable fungus, easily mutated with different colony growth colors when subcultured on the same medium (medium rich in carbohydrates). Unlike the genus *Trichoderma* sp., the genus *Fusarium* sp. is not easy to recognize if you only observe the color of the growth of the colony.

Macroscopic and microscopic observations of isolates PR1, PR2, PR3 according to the genus *Penicillium* sp. The colors produced from the three isolates were the same, namely light green with a cloudy white border (observed from the upper surface) while the bottom was cream, cloudy white and gray. The isolates had hyaline and insulated hyphae, the conidia shape of the three isolates was small round (Table 2, Figure 3). Hyphae are septate, branched mycelium is usually colorless, conidia oval shape is a specific feature of the fungus *Penicillium* sp. (Ratnawati et al. 2020; Hidayah et al. 2021).

The color of the colony appears above is dark brownish green, the texture is thick and flat, the color is cloudy white below, the hyphae are hyaline and insulated, and the conidia are small round dispersed which are the characteristics of the isolates GR1 and GR2. The isolates were identified in the *Gliocladium* sp. In line with research (Lestari et al. 2021) that *Gliocladium* sp. has conidia with round shape and green spore spots. Naturally, *Gliocladium* sp. can produce antibiotics which have been widely used as biological control agents for pests (Hassine et al. 2022).

The isolates of the fungus AR were identified in the genus *Aspergillus* sp. which were observed macroscopically and microscopically. This isolate has the characteristics of black colony growth in the middle with white edges, flat colony edges, thick and rough texture that appears on the upper surface, the bottom is cloudy white. Microscopically this isolate had hyaline hyphae, septate, with an unbranched coniodophor shape, while the conidia were small round. Colony color is generally influenced by the color of the spores (Qayyum et al. 2021). This fungus is found in the soil, especially soil that contains a lot of high organic matter so that it has a wide distribution (Frac et al. 2018).

Poso and Sigi Regencies, Central Sulawesi Province store a lot of microbial diversity that can be found in two different ecosystems, including lowland rice ecosystems and onion plantations. The results of the inventory that had been carried out were obtained as many as 12 bacterial isolates from lowland rice cultivation and 16 fungal isolates from the shallot plantation "Lembah Palu" were identified morphologically, macroscopically and microscopically. Bacterial isolates had different characteristics based on morphological and physiological characterization tests, while fungal isolates were identified into 5 genera, namely *Trichoderma, Fusarium, Penicillium, Gliocladium* and *Aspergillus.*

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