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EXPLORATION AND IDENTIFICATION OF MICROBES AND FUNGI FROM SOIL SAMPLES OF GUNGGUNG VILLAGE, BATUAN DISTRICT, SUMENEP REGENCY AS BIOLOGICAL CONTROL AGENTS AT THE EAST JAVA FOOD CROPS AND HORTICULTURE PLANT PROTECTION LABORATORY

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Abstract

Soil microorganisms play an essential role in maintaining soil fertility and suppressing plant pathogens, making them potential biological control agents in sustainable agriculture. This study aimed to explore and identify microbes and fungi from soil samples collected from yellow Kepok banana (Musa spp.) plantations in Gunggung Village, Batuan District, Sumenep Regency, as potential biological agents. The novelty of this research lies in the exploration of untreated Fusarium-infected banana plantation soil to obtain indigenous microbial isolates with biocontrol potential. A descriptive exploratory research design was employed. Bacterial isolation was conducted using the serial dilution method on Potato Dextrose Agar (PDA), followed by purification, macroscopic and microscopic characterization, 5% KOH test, soft rot test, and hypersensitivity test. Fungal isolation was performed using the baiting method with coconut, rice, and mealworms as substrates, followed by morphological identification. The results revealed the presence of Bacillus sp. as the dominant bacterial isolate, characterized by circular colonies, yellowish-white color, Gram-positive reaction, and rod-shaped cells. Soft rot and hypersensitivity assays indicated varying pathogenic responses among isolates. Fungal identification showed the presence of Aspergillus sp. (brownish-green, powdery colonies) and Penicillium sp. (yellowish-green, granular colonies). These findings indicate that soil from the study site contains microbial populations with potential applications as biological control agents. The study highlights the importance of exploring indigenous soil microorganisms to support environmentally friendly plant disease management and sustainable agricultural practices.

Keywords : *Identification, Microbes, Fungi, Biological Agents*.

INTRODUCTION

Microorganisms are microscopic organisms such as bacteria, fungi, algae, viruses, and protozoa that play an important role in maintaining ecosystem balance and supporting agricultural productivity. In soil, microorganisms function as decomposers that break down organic matter, increase nutrient availability, and produce bioactive compounds beneficial to other organisms (Agastya et al., 2018). In addition, microorganisms can be utilized as antagonistic agents to inhibit the growth of pathogens through biological mechanisms that are more environmentally friendly than synthetic pesticides (Khairani et al., 2025). The appropriate use of microorganisms also contributes to the control of plant pests and diseases (PPD) and to the improvement of soil health (Sutanto et al., 2023).

Plant pests and diseases (PPD) are among the main factors causing a decline in agricultural productivity due to attacks by insects, bacteria, fungi, and viruses. The damage caused can be physical, physiological, and biochemical, making their management a crucial aspect of agricultural production systems (Serealia et al., 2023). To date, PPD control is still dominated by the use of synthetic pesticides, although excessive application can lead to resistance, pest resurgence, and environmental pollution (Efikasi et al., 2013). Therefore, biological control based on antagonistic microorganisms has become an effective and sustainable alternative to suppress the development of plant pathogens (Lestari et al., 2021).

The development of biological control concepts is supported by numerous studies demonstrating the effectiveness of microorganisms such as *Pseudomonas* sp., *Bacillus* sp., *Burkholderia* sp., and *Trichoderma* sp. in inhibiting soil-borne and foliar pathogens (Abdelaziz et al., 2023). *Trichoderma* sp. is known to possess diverse biocontrol mechanisms, both direct and indirect, and is therefore widely developed as a biological agent in sustainable agriculture (Heikal, 2024). On the other hand, soil microorganisms also play a role in maintaining soil health and coping with environmental stresses such as increased salinity, which can affect the functioning of agricultural ecosystems (Zhang *et al.*, 2024; (Dohare et al., 2025).

Based on this urgency, the exploration and identification of microbes and fungi from soil samples are strategic steps to discover potential biological agents that are effective, safe, and environmentally friendly. The isolation and identification of soil bacteria and fungi enable the acquisition of pure cultures with potential applications as biofertilizers, plant disease control agents, and producers of bioactive compounds (Istighfari *et al*., 2024; (Umboh et al., 2024). Therefore, research on the exploration and identification of microbes and fungi from soil samples in Gunggung Village, Batuan District, Sumenep Regency, conducted at the Laboratory of the East Java Food Crops and Horticulture Plant Protection Unit (UPT Proteksi Tanaman Pangan dan Hortikultura Jawa Timur), is important to support the development of biological agents and sustainable agricultural practices.

METHOD

This study employed a descriptive exploratory research design. Descriptive exploratory research involves conducting field exploration activities followed by laboratory procedures to develop fungal and bacterial isolates. Fungi were cultured using the baiting method, whereas bacteria were developed using the dilution method.

The history of the land from which the soil samples were collected indicates that it had been cultivated with yellow Kepok banana (*Musa* sp.). Banana plants belong to the family Musaceae and contain vitamin C, B-complex vitamins, vitamin B6, and serotonin, which functions as an important neurotransmitter supporting optimal brain function (Triwidodo et al., 2020).

The yellow Kepok banana plantation showed that the plant pest and disease (PPD) attacking the crop was *Fusarium* sp. Symptoms of Fusarium wilt observed in the field included wilting and yellowing of the plants. Yellow spots or streaks appeared at the base of the leaves, while the lower leaf margins turned dark yellow, then brown, and eventually became dry and brittle. The leaf sheaths could also break, and the pseudostem occasionally split (Lea et al., 2023). The samples were collected from 12-

month-old banana plants. To date, no treatment has been applied to the land.

Soil samples were collected from the Sumenep area. The isolation and identification of the obtained fungi and bacteria were conducted at the Biological Control Agents Laboratory of the Food Crops and Horticulture Plant Protection Unit (UPT) in Surabaya. The research was carried out from February 3 to May 8, 2025, over a period of three months.

Sample plastic bags, test tubes, Bunsen burner, laminar air flow cabinet, measuring cylinder, inoculating loop, dropper pipette, heat-resistant plastic, autoclave, camera, balance, stationery, aluminum foil, plastic wrap, labeling paper, trays, jars, plastic containers, black cloth, Petri dishes, Erlenmeyer flasks, test tube rack, microscope, and syringe. Materials: Soil samples from cultivated areas (banana plantations), potatoes, agar, dextrose, 70% alcohol, distilled water (aquades), mealworms (*Tenebrio molitor*), and rice.

Sterilization of equipment and materials was performed using a semi-autoclave, which was tightly sealed and checked to ensure that the pressure system was properly closed. Initial heating was carried out using a stove over medium heat for 15 minutes. The heat was then turned off, the steam valve was opened, and the steam was allowed to escape completely to remove residual contaminated steam. After releasing the steam, the semi-autoclave was closed again and reheated until the pressure gauge indicated 1 atm. When the pressure reached 1 atm (121°C), sterilization was conducted for 60 minutes for equipment and 30 minutes for materials to eliminate microorganisms. Finally, after the sterilization period was completed, the heat was turned off, the steam valve was opened, and the semi-autoclave was allowed to return to normal pressure before being opened.

The soil sample dilution process was carried out using the serial dilution method to obtain a representative microbial suspension. A total of 10 g of finely ground soil sample was added to 100 mL of sterile distilled water in an Erlenmeyer flask and homogenized to form an initial suspension. Seven test tubes were prepared, each containing 9 mL of sterile distilled water and labeled 10^{-1} to 10^{-7}. One milliliter of the initial suspension was inoculated into the 10^{-1} tube and homogenized using a vortex mixer. Serial dilutions were then performed by transferring 1 mL from each tube to the next until a dilution level of 10^{-7} was achieved, with homogenization carried out at each step. From the entire dilution series, suspensions at dilution levels of 10^{-4}, 10^{-5}, and 10^{-6} were selected for subsequent isolation and analysis.

The inoculation process was carried out after the dilution stage using Potato Dextrose Agar (PDA) medium in six sterile Petri dishes. Suspensions from dilution levels of 10^{-4}, 10^{-5}, and 10^{-6} were each taken in three drops using a sterile syringe, then dispensed onto the surface of the PDA medium and evenly spread using a triangular spreader (spread plate method). Each dilution level was performed in duplicate to enhance the validity of the results. After spreading, the Petri dishes were closed and sealed with plastic wrap to prevent contamination. All samples were then incubated for 2 × 24 hours to allow microbial colony growth.

Purification was conducted within 2 × 24 hours after inoculation, once single colonies had appeared. The single colonies observed on the Petri dishes were selected and further purified under a laminar air flow (LAF) cabinet. Each single colony was picked using a sterile inoculating loop and streaked onto a Petri dish containing PDA medium using the four-quadrant streak method. The plates were then sealed with plastic wrap and incubated for 2 × 24 hours to observe the bacterial growth.

Isolation of soil samples using the baiting method was conducted in several stages. First, the required materials were prepared, including three plastic containers and 500–600 g of soil samples collected from the field that had been affected by pest infestation. Fungal baits consisted of corn, rice, and mealworms. In the second stage, three plastic containers were prepared, and the soil sample was divided into three equal portions and placed into each container. The prepared baits were then placed into the containers containing the soil samples. The containers were subsequently covered and incubated until fungal growth was observed.

The Gram method using a 3% KOH test is an effective bacterial identification technique to determine the dominant active bacterial type, which is indicated by the formation of a viscous thread. If the amount of bacterial culture used is insufficient, the test may yield inaccurate results

and fail to show a reaction; therefore, the use of a dark-colored background is highly recommended during the procedure (Hardiansyah et al., 2020). The soft rot test was used to identify whether the bacteria exhibited parasitic or saprophytic characteristics, using potato as the test medium. Antagonistic microbes are generally obligate saprophytes and can only grow on dead tissue. If the potato undergoes decay after inoculation with the bacterial isolate, it can be presumed that the bacterium exhibits parasitic propertie (Made, 2023). The hypersensitivity test is conducted to determine whether the tested bacterial isolate is pathogenic or non-pathogenic. This assay is performed by inoculating a bacterial suspension into tobacco plant tissue. A positive reaction is indicated by wilting or even necrosis of the tobacco tissue at the site of bacterial inoculation (Ekwueme & Alike, 2024).

The data used in this study were obtained from field exploration and the identification of microbes and fungi after isolation at the Biological Control Agents Laboratory in Surabaya. These data included the morphological features and characteristics of the isolated microbes, and fungi.

RESULTS

The soil samples were obtained from a yellow Kepok banana plantation in Sumenep that was infected with *Fusarium* sp. and had not received any treatment. *Fusarium* sp. can spread by infecting plant roots in the form of mycelium. Infection in nurseries causes the shoots to wilt and eventually die. In mature plants, the upper leaves become pale while the lower leaves turn yellow, followed by downward curling of the leaf tips until the plant eventually dies. Symptoms of Fusarium wilt begin at the lower part of the plant and progress upward. When observed in cross-section, the main stem shows brown discoloration in the vascular tissues (Raharjo et al., 2017). Following identification tests, bacteria were also detected in the soil samples.

Bacterial Eksploration

Bacteria are microorganisms that live and function in environments containing hydrocarbons. Microbes capable of degrading hydrocarbons are widely distributed in various environments and proliferate when conditions are favorable. One example is *Bacillus* sp. Based on the exploration results of bacterial isolates from Sumenep soil samples, both macroscopic and microscopic observations were conducted.

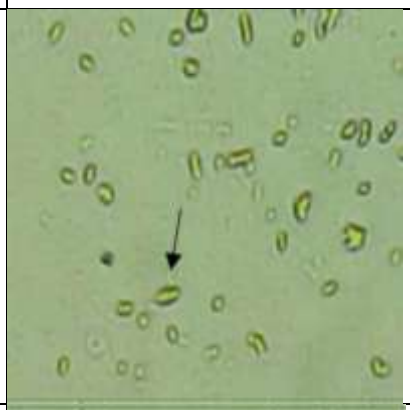
Table 1 **Macroscopic Identification Results of Bacteria**

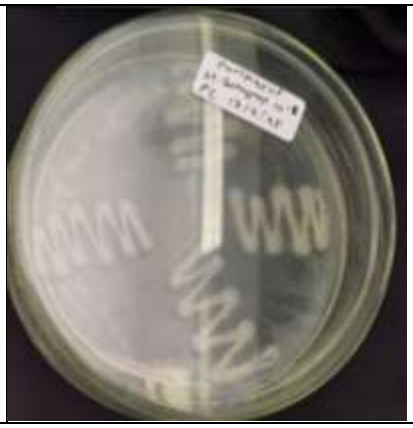
No	Isolate Code	Colony Surface	Colony Color	Colony Margin	Colony Shape
1	A	Raised	Yellowish White	Entire	Circular
2	B	Flat	Yellowish White	Entire	Circular
3	C	Flat	Yellowish White	Undulate	Circular
4	D	Raised	Yellowish White	Entire	Circular

In the banana soil samples (Table 1), the bacterial colonies were observed to have a circular shape. The colonies exhibited a yellowish-white color, with entire and undulate margins. The colony surface was either raised or flat. Endophytic bacteria *Bacillus siamensis* and *Bacillus subtilis* were identified to verify the bacterial isolates used in this study. Identification results indicated that both *Bacillus siamensis* and *Bacillus subtilis* displayed similar colony morphology. Both species produced cream or yellowish-white colonies with a circular shape, rough surface texture, convex elevation, and entire colony margins (Aktivitas et al., 2021). *Bacillus* sp. is capable of forming endospores as a survival mechanism, enabling the organism to withstand unfavorable environmental conditions such as extreme temperatures, drought, and nutrient deficiency. Several species within the genus *Bacillus* function as pesticides, fungicides, biofertilizers, and also possess plant growth-promoting abilities (Wulandari et al., 2019).

Microscopic and Macroscopic Characterization of Isolates

Table 2 ***Macroscopic and Microscopic Observations of Bacteria at 40× Magnification***

No.	Kode isolat	Macroskopic	Microskopic
1.	A		
2.	B		
3.	C		

4.	D		

The results of macroscopic observation showed that the bacterial colonies had either flat or raised surfaces. Microscopic observation was conducted to examine bacterial cells that could not be observed with the naked eye. As presented in Table 2, the bacterial colonies exhibited a bacillus (rod-shaped) form. The cells were oval in shape with a diameter of 2.0 mm and displayed a yellowish-white color.

5% KOH Test Observation Results

Table 3 ***5% KOH Test Results of Bacterial Colonies***

No	Kode isolat	Uji KOH
1	A	Reaction (-). Gram (+)
2	B	Reaction (-). Gram (+)
3	C	Reaction (-). Gram (+)
4	D	Reaction (-). Gram (+)

Description:

Gram Test: (-) sign indicates a Gram-negative test result with a positive reaction, while the (+) sign indicates a Gram-positive test result with a negative reaction.

Based on the test results presented in Table 3, according to (Agensia & Hayati, 2023), *Bacillus* sp. subjected to the KOH test showed that Gram-positive bacteria do not produce a viscous thread because their cell walls contain a thick peptidoglycan layer (approximately 90%). The results of testing the bacterial isolates on potato tubers over three days of observation showed symptoms of soft rot, characterized by tissue softening, the appearance of slime on the infected areas, discoloration of the tubers to yellowish-brown, and an unpleasant odor as the symptoms progressed. These findings are consistent with those reported by (Husen *et al*., 2018).

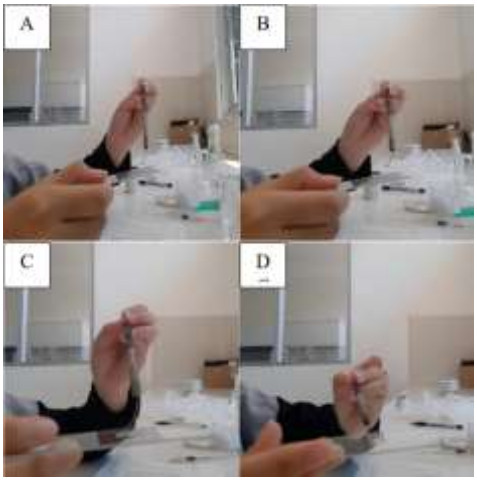


Figure 1 ***5% KOH Test Results of Isolates A), B), C), and D)***

Soft Rot Test Results

Table 4 ***Soft Rot Test Results of Bacterial Colonies***

No	Kode Isolat	Soft Rot Test
1	A	+
2	B	+
3	C	+
4	D	+

Description:
Soft Rot Test: The (+) sign indicates that the bacterium is capable of causing decay in potato tubers, whereas the (−) sign indicates that the bacterium does not cause decay in potato tubers.

The results of the soft rot test observation (Figure 2) showed that the wounded and pathogen-inoculated areas appeared decayed and formed deposits. When the potato tubers were cut open, they exhibited soft rot symptoms characterized by tissue maceration and slime formation, with the decay progressively spreading. The soft rot test is used to determine whether bacteria exhibit parasitic or saprophytic characteristics and is performed using potato as the test medium. Antagonistic microbes are generally obligate saprophytes and can only survive on dead tissue. If decay occurs in the potato after inoculation with a bacterial isolate, it is likely that the bacterium is parasitic (Saadah *et al*., 2023). This indicates that the disease suppression mechanism may be attributed to antagonistic bacterial isolates that produce secondary metabolites capable of inhibiting pathogen development. In addition, disease suppression may also occur because the tested antagonistic bacterial isolates enhance the resistance of potato tubers. Numerous studies have reported the ability of antagonistic bacteria to induce plant resistance against diseases (Paisal *et al*., 2023).

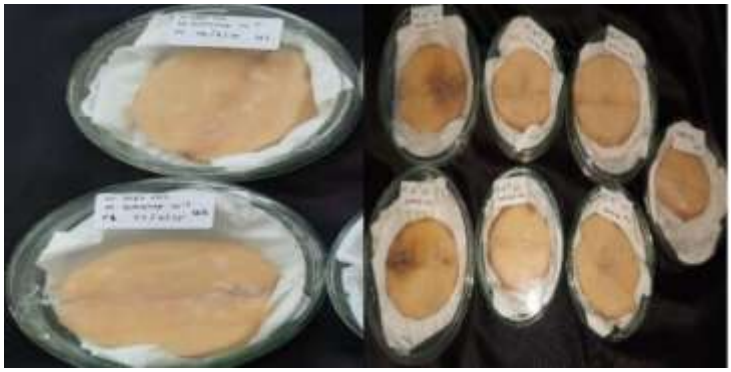


Figure 2 ***Results of the Soft Rot Test***

Hypersensitivity Test Results

Table 5 ***Results of the Hypersensitivity Assay***

No	Kode Isolat	Hypersensitivity Test
1	A	+
2	B	-
3	C	-
4	D	-

Description:

Hypersensitivity Test: The (+) sign indicates that the bacterium is pathogenic, whereas the (−) sign indicates that the bacterium is non-pathogenic.

Based on the results shown in Figure 3, one sample exhibited necrosis, while the other three samples showed no necrotic symptoms. The endophytic bacterial suspension was collected using a sterile syringe and injected into the abaxial (lower) surface of tobacco leaves, followed by incubation for 48 hours. Bacteria with pathogenic potential exhibited necrotic symptoms (positive reaction) on tobacco leaves, whereas non-pathogenic bacteria did not cause necrosis (Marsaoli & Matinahoru, 2019). *Bacillus* sp. is classified as a biological control agent for managing Fusarium disease in the soil samples collected from yellow Kepok banana plantations. Both *Bacillus* sp. and *Pseudomonas* sp. are recognized for their potential as biological control agents against various plant pathogens. Their effectiveness as biological agents is associated with their ability to compete for nutrients and produce secondary metabolites such as antibiotics, siderophores, and extracellular enzyme (Diarta et al., 2016). Furthermore, *Bacillus* spp. can produce phytohormones that enhance plant growth and facilitate the uptake of certain nutrients from the environment. *Bacillus* spp. influence plant growth through both direct and indirect mechanisms. Directly, rhizobacteria provide compounds synthesized by *Bacillus* sp., such as phytohormones, or facilitate nutrient absorption. Indirectly, they induce systemic resistance, characterized by the accumulation of salicylic acid and pathogenesis-related protein (Udayana et al., 2022).

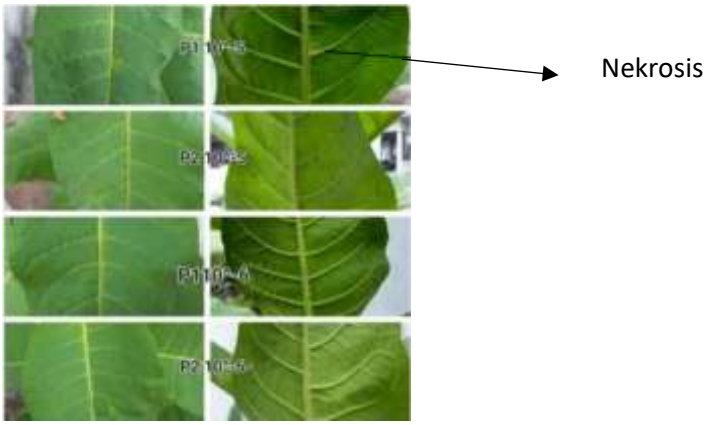


Figure 3 Hypersensitivity Test

Fungal Exploration

Based on the results obtained from the yellow Kepok banana soil samples, a variety of fungi were identified. The soil samples were subjected to three treatments using mealworms, rice, and coconut as baiting materials. The isolated fungi were then observed both macroscopically and microscopically.

Results of Macroscopic Observation of Fungi

Table 6 ***Results of Macroscopic Identification of Fungi from the Baiting Method***

No	Treatment Type	Genus	Color	Colony Texture
1	Coconut	*Aspergillus* sp.	Brownish green	Powdery
2	Rice	*Aspergillus* sp.	Brownish green	Powdery
3	Rice	*Penicillium*	Yellowish Green	Granular

The results from (Table 6) show that in soil samples treated with coconut and rice, fungi identified as *Aspergillus* sp. were observed. *Aspergillus* sp. is a saprophytic fungus that can be found in various environments, such as soil, the open air, and food materials including rice, wheat, peanuts, oncom, tempeh bongkrek, as well as canned foods like corned beef and sardines. *Aspergillus flavus* forms colonies with colors ranging from yellow-green and yellow-gray to black. Its conidiophores are colorless, rough, with slightly rounded tips, while the conidia produced are rough and display a variety of colors. Foods we consume are highly susceptible to contamination by *Aspergillus flavus* (Amalia, 2013). Species of *Aspergillus* are fungi capable of solubilizing phosphate, including phosphate from sources that are otherwise difficult to dissolve. This fungus can also solubilize insoluble inorganic phosphate by producing organic acids (Putu et al., 2018).

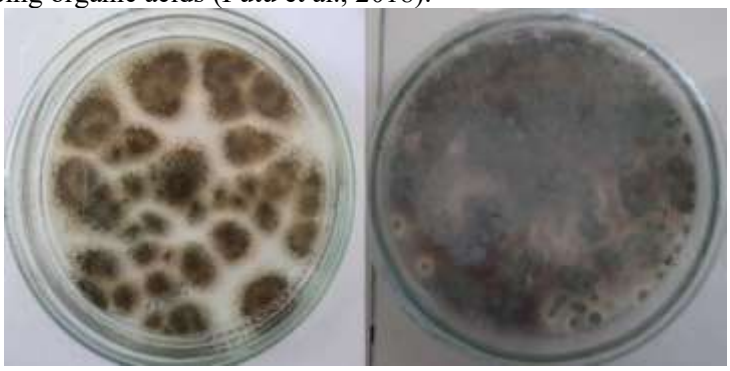


Figure 4 ***A)*** **Aspergillus** ***sp. and B) P*****enicillium** ***sp.***

Microscopic Observation Results of Fungi

The results of the observation (Figure 5) from the baiting showed that the growth of *Aspergillus* requires nutrient sources such as carbohydrates, minerals, and fats from coconut. To identify *Aspergillus*

species microscopically, several characteristics need to be observed, including the shape of the conidial head, the shape and diameter of the vesicle, conidia size, texture, and color. The isolation results revealed variations in these characteristics. This observation is in line with the study by (Nyongesa et al., 2015), which stated that the macroscopic morphology of *Aspergillus* species includes colony texture, pigmentation, color beneath the colony, and the formation of sclerotia consisting of exudate droplets, radial grooves, and growth zones.

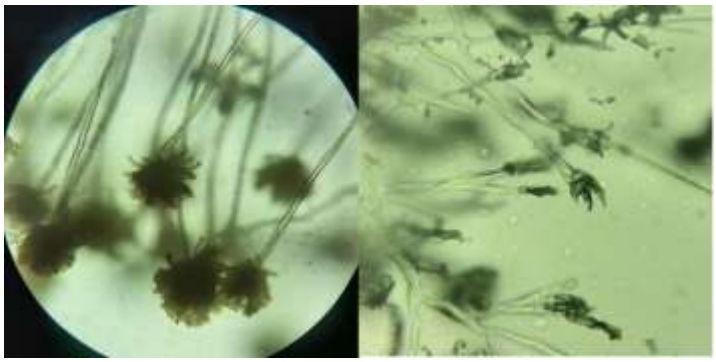


Figure 5 *A)* Aspergillus *sp. B)* Penicillium *sp.*

Aspergillus sp. is a fungus belonging to the class Ascomycetes and can be found in various natural environments. This fungus grows as a saprophyte on decaying plants. Its macroscopic characteristics include colonies that are green in the center and white at the edges, organized mycelium, colonies that grow flat and thick, and flat colony margins. Other macroscopic features include aseptate hyphae, branched mycelium, upright and elongated conidiophores with swollen tips forming vesicles (Sakiah et al., 2024).

Aspergillus sp. is a genus of anamorphic (asexual) fungi that reproduces by producing phialospores (conidia that grow from phialides). This genus can be recognized by its characteristic conidiophores, which are arranged in either uniseriate or biseriate patterns. Most *Aspergillus* species grow well at temperatures between 27–37°C and across a wide pH range. *Aspergillus* is also known for its ability to produce various enzymes, such as cellulase, amylase, glucoamylase, lipase, and protease, as reported in previous studies. *Aspergillus* fungi can be isolated from soil and decaying organic matter without requiring special techniques, as they can produce spores abundantly and germinate rapidly on general or selective media (Sari1 et al., 2017). *Aspergillus niger* has long been recognized as a microorganism with remarkable capability in producing various enzymes with important industrial applications. Its advantage over other microorganisms lies in its ability to produce cellulase, particularly β-glucosidase, in significant amounts. During metabolism, *Aspergillus niger* can produce weak organic acids, such as citric acid, oxalic acid, and gluconic acid. Some important enzymes produced by *Aspergillus niger* with industrial applications include amylase, cellulase, and amyloglucosidase (Wisda et al., 2016).

The results of the observation showed that *Penicillium* sp. grew on rice bait because rice provides the nutrients required by the fungus. Macroscopic observation of the fungal samples on PDA media indicated that the colonies were dark green in the center and white at the edges. The colonies were circular, with a smooth texture, raised elevation, and overall rounded margins. Microscopic observations revealed hyphae that were hyaline in color. The conidiophores were branched, forming several phialides at their tips. The conidia were hyaline, growing at the tips of the phialides, spherical in shape, and forming long chains. *Penicillium* sp. has conidiophores that grow from the mycelium, with branched tips forming clusters of phialides. The conidia produced are hyaline or bright, mostly ovoid, and arranged in long chains. *Penicillium* sp. belongs to the class Eurotiomycetes, order Eurotiales, and family Trichocomaceae (Mahendra et al., 2022). *Penicillium* is a type of fungus commonly found in various environments, such as soil, marine habitats, food, and even as endophytes in plants. This fungus is widely utilized in industry, including for the production of antibiotics, enzymes, pigments, and as a fermentation agent in food products such as cheese (Aji et al.,

2021). Endophytic fungi are fungi that live within plant tissues, such as leaf tissue. They can be found in various plant tissue systems, including leaves, flowers, branches, and roots. These fungi infect specific parts of healthy plants and are capable of producing mycotoxins, enzymes, and antibiotics (Eko et al, 2013).

CONCLUSION

Based on the results obtained from the exploration and identification of bacteria and fungi in soil samples from Sumenep conducted at the Biological Agents Laboratory, UPT Plant Protection for Food Crops and Horticulture, Surabaya, the following conclusions can be drawn: The exploration and identification of bacteria from Sumenep soil samples at the Biological Agents Laboratory, UPT Plant Protection for Food Crops and Horticulture, revealed the presence of *Bacillus* sp. For fungi, *Aspergillus* sp. and *Penicillium* sp. were identified from the soil samples of yellow Kepok banana (*Musa* spp.) commodities in Sumenep. The characteristics of *Bacillus* sp. include macroscopic colony surfaces that are either raised or flat, with colony colors ranging from white to yellowish. Microscopically, the cells are bacillus-shaped with oval forms. The macroscopic characteristics of *Aspergillus* sp. include greenish-brown coloration with a powdery colony texture. *Penicillium* sp., found on rice bait, exhibits macroscopic characteristics of yellowish-green color and granular colony texture.

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