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RESEARCH ARTICLE

ISOLATION AND MORPHOLOGICAL IDENTIFICATION OF SOIL FUNGI FROM AGRICULTURAL SOIL IN KUANTAN

Nur Sabrina Ahmad Azmi*, Asma Adiba Hisham

Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia
****Corresponding author email: sabrinaazmi@iium.edu.my***

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ABSTRACT

Soil fungi possess a great number of potential benefits that could be applied in various fields. They are well-known for acting as plant-growth promoter, biocontrol agent of plant diseases and involves in bioremediation. In this study, the fungi were isolated from used agricultural soil in Glasshouse and Nursery Complex (GNC), International Islamic University Malaysia, Kuantan, Pahang using serial dilution and plating techniques. Around 10 isolates of soil fungi were successfully isolated and the identification of all isolates were based on their cultural and morphological characteristics. The fungi were discovered to be from genus *Cladorrhinum, Penicillium, Paecilomyces* and *Aspergillus*.

KEYWORDS

Soil fungi, *Cladorrhinum, Penicillium, Paecilomyces, Aspergillus*, agricultural soil.

1. INTRODUCTION

Soil is the outer layer of the Earth's crust which is loosely-arranged and has many forms (Baxter and Williamson, 2001). It generally consists of organic and inorganic matter. The organic fraction of soil is divided into biomass that includes living organisms and necromass which is the dead organisms and their transformation products (Nortcliff et al., 2006). A gram of soil is estimated to accommodates around 90–100 million bacteria and about 200 000 fungi, with most of these organisms being located at the rhizosphere (Glick, 2018). These organisms play an important role in maintaining soil health, quality and fertility through their natural interactions and processes (Lori et al., 2017).

The physical, chemical and biological properties of soil significantly affect the production and sustainability of agricultural practices. This is because the soil acts as a medium for plant growth which stores nutrients, water and also provides an environment for the breakdown and immobilization of materials such as fertilizers (Nortcliff et al., 2006). Growing media mixes mainly includes topsoil, sawdust, perlite, vermiculite, peat moss, bark and composts (Gruda, 2019). Topsoil is recognized as the outermost few centimetres or more of soil which is rich with organic matter and microorganisms (Darmody et al., 2009). Agricultural activities cause both good and bad effects to soil organisms. For instance, the application of raw and composted organics as fertilizer promote microbial proliferation in the soil (Bunemann et al., 2006).

However, agricultural management such as soil tillage had been found to decrease the population of soil organisms due to mechanical destruction and soil compaction (Garcia-Orenes et al., 2013). Besides, the extensive use of synthetic mineral nitrogen source as fertilizer negatively influenced the soil microorganisms' communities and diversity (Gryndler et al., 2006). Following these practices, an effective method of soil management is therefore vital in order to maintain crop productivity, environmental sustainability, and human health. Due to the steady increase of world population and thus escalating food production needs, proper actions should be taken to overcome this problem in the coming years.

One of the methods being taken into action as a solution is practicing sustainable agriculture. This includes all of the systems and practices that will improve the protection of the environment and natural agricultural resources necessary to ensure the production of adequate and high-quality foodstuffs at affordable costs which the rapidly growing world population needs (Tuğrul, 2020). It mainly focuses on increasing the productivity of the soil and reducing the harmful effects of agricultural practices on climate, soil, water, environment and human health.

The decrease of arable land and resources had become the major reasons for the need to have sustainable agricultural practices. However, most of the agricultural activities nowadays have been causing the quality of soil health to decrease especially by affecting the soil microbiome. The soil fungal species have both good and bad impacts on agriculture. Therefore, the isolation and identification of soil fungi is important to further characterize their functions in soil ecosystem. Therefore, the aim of this study are i) to isolate soil fungi communities from used agricultural soil in Glasshouse and Nursery Complex, IIUM using serial dilution and plating techniques and ii) to identify the species of isolated soil fungi based on their cultural and morphological characteristics.

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2. Materials and Methods

2.1 Sample collection

Soil sample was collected from the used agricultural soil in Glasshouse and Nursery Complex (GNC), IIUM in September 2019. The soil sample has been used by the students for laboratory practicals or experiment purposes, which is a mixture of top soil, peat moss and sand. Firstly, the soil sample was air-dried for 3 days in the laminar air flow and was ground by using mortar and pestle. Ten grams of the dried soil was weighed.

2.2 Preparation of soil dilution and microbial culture

Isolation of soil fungi by soil dilution plating was adapted from method by Pepper and Gerba (2004). Ten grams of the soil sample was added to 95 mL of deionized water (solution A) and shaken well to disperse the organism. This yielded a 10-fold dilution series. Next, 1 mL of the suspension was removed from solution A using a sterile pipette and then transferred to a new tube containing 9 mL dilution blank (solution B). The tube was capped and vortexed. The dilution factor for solution B is 1×10^{-2}. The dilution series was continued to obtain solutions with dilution factors of 1×10^{-3} (solution C), 1×10^{-4} (solution D) and 1×10^{-5} (solution E). Next, 1 mL of each soil dilution was transferred into petri dish containing molten rose bengal agar supplemented with 25 µg/mL chloramphenicol. The medium and inoculum were mixed before the agar solidifies. After all plates have solidified, they were incubated at 28˚C for 1 week.

2.3 Isolation of fungi

The presence of fungi isolates were observed every day for 7 to 10 days. The fungi were then separated based on color and surface morphology to Potato Dextrose agar (PDA) plates with each type of fungi was transferred to 3 plates. Next, the plates were incubated at room temperature for 7 days. Then, in order to obtain the pure culture, the fungi colony formed were inoculated on water agar for 5 to 7 days to isolate single spore or hyphal tip. The single spore or hyphal tip for each isolate was then inoculated on new PDA as the pure culture. The pure culture fungi were inoculated on slanted PDA for long term storage.

2.4 Cultural, morphological and microscopic identification of soil fungi isolates

For cultural and morphological characteristics of the fungi, colony color, texture, diameter, hyphae and conidia were observed. As for microscopic identification, the slides were prepared and examined using compound and light microscope (Leica ICC50 HD) equipped with LAS EZ software. Different genera are identified using identification key from Pictorial Atlas of Soil and Seed Fungi (Watanabe, 2010).

3. Results

3.1 Cultural, morphological and microscopic characterization of soil fungi isolates

A total of 10 fungi isolates were obtained from the used agricultural soil in GNC IIUM and were identified based on their cultural and morphological characteristics such as colony color, reverse color, texture, margin, presence of sulcation and colony diameter. The fungi isolates obtained can be grouped into 4 genus based on their morphological characteristics which are *Cladorrhinum sp., Paecilomyces sp., Aspergillus sp. and Penicillium sp.* suggesting that the diversity of fungal population in the used agricultural soil.

3.2 *Cladorrhinum* sp.

Three isolates of fungi (IIUM C1, IIUM C2 and IIUM E13) exhibit white to pale cream colony on PDA with a cottony texture except for IIUM E13 which demonstrate velvety texture. The colony margin is smooth (IIUM C1 and IIUM C2) and filamentous (IIUM E13) with the diameter range between 3.0 cm- 3.6 cm. The colonies exhibit septate hyphae and the conidia are phialospore-type, 1 celled, hyaline, globose with conidiophores are without an inflated apical cell (Figure 1).

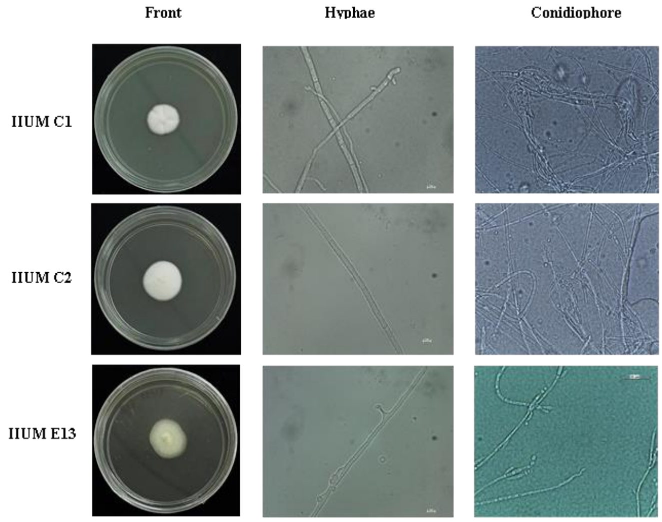

Figure 1: Cultural and morphological characteristics of *Cladorrhinum* sp.

3.3 *Paecilomyces* sp.

All colonies demonstrate white color with some exhibit pale green at the center on PDA. The colonies shows cottony texture and filamentous margin with a diameter range in between 3.5 cm- 4.2 cm. The conidia are phialospore-type, 1 celled, aggregate in a row with limoniform structure; and conidiophores are well developed without an inflated apical cell and hyaline (Figure 2).

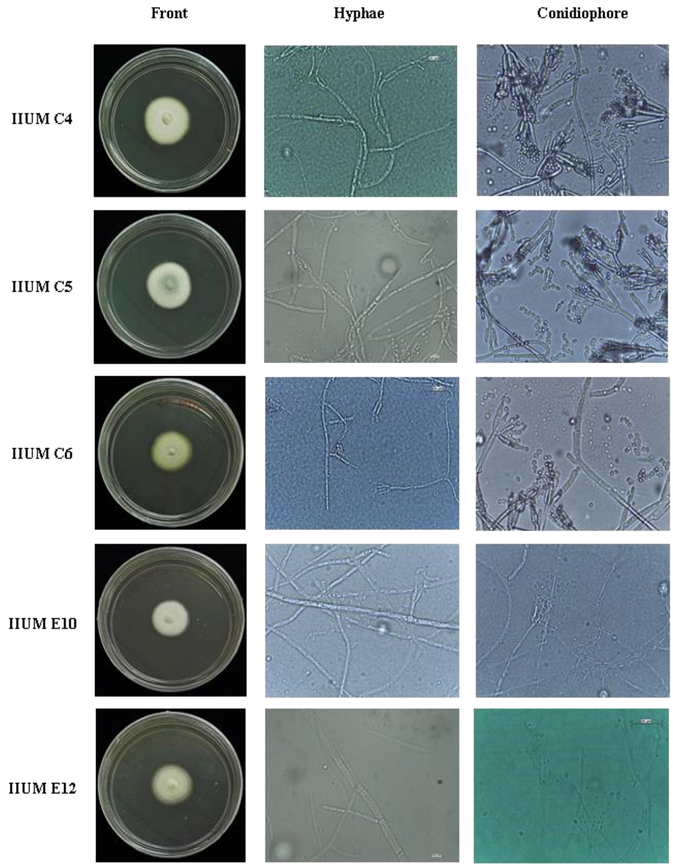

Figure 2: Cultural and morphological characteristics of *Paecilomyces* sp.

3.4 *Aspergillus* sp.

The colony is white yellowish on PDA with a granular texture and filamentous margin. It is a fast growing fungi with the colony diameter range between 6.5 cm to 7.0 cm at 7 days. The hyphae is septate and conidiophores are biseriate with apically inflated globose, and bearing numerous phialides (Figure 3).

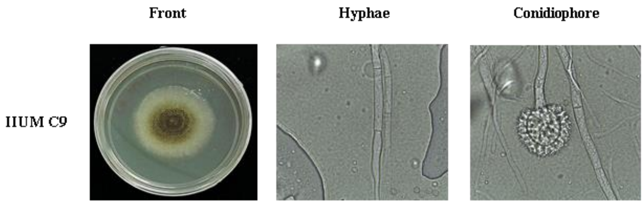

Figure 3: Cultural and morphological characteristics of *Aspergillus* sp.

3.5 *Penicillium* sp.

Colony is green with white margin on PDA with a velvety texture and smooth margin. The colony is 3.0 cm to 3.2 cm in diameter. The colony shows the presence of septate hyphae and the conidia are phialospore-type, 1 celled, dry, and globose, with a well-developed penicillus and conidiophores are well developed without an inflated apical cell, hyaline and the spores are aggregated in a row (Figure 4).

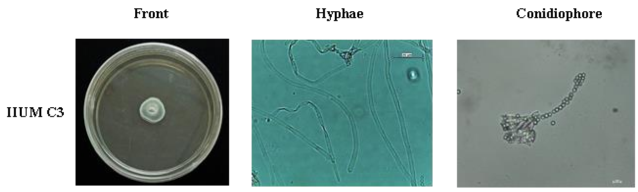

Figure 4: Cultural and morphological characteristics of *Penicillium* sp.

4. Discussion

The used agricultural soils in GNC, IIUM harbors many potential microbes that can be further explored for their effectiveness in agriculture and many other industries. One of the significant ability of fungi belonging to the genus *Cladorrhinum* is their biocontrol potential against plant diseases. Since the awareness of the society on environmental conservation is on the rise, the use of microbial-based fungicide showed an increase in significance as an alternative to chemical products which will inevitably cause negative impacts on the environment. The study of fungi from genus *Cladorrhinum* by found that it has the ability to reduce disease incidence in cotton crops infested by *Rhizoctonia solani* (Gasoni and Gurfinkel, 2009). In addition, cotton plants growing in phosphorus deficient substrate showed a significant increase in plant height and phosphorus content at blossom stage when the root were colonized with *C. feocundissimum* (Gasoni and Gurfinkel, 1997).

This proved that the infestation by *C. foecundissimum* could increase the availability of phosphorus for root uptake. However, the mechanisms on how the fungi enhance nutrient uptake are yet to be known. Aside of *Cladorrhinum* sp., the biocontrol ability of some *Aspergillus* sp is also notable. For instance, 3 *Aspergillus* isolates inhibited the growth of *Fusarium oxysporum*, a causal agent of *Fusarium* wilt in banana (Hidayat et al., 2019). Aside of that, *Aspergillus* strains were found to have reduce mycelial growth of *F. sambucinum* and *Phytophthora erythroseptica* by 27% to 68% and 16% to 25% respectively, therefore having potential to be used as natural fungicide (Abdallah et al., 2015). In addition, the inoculation of *A. niger* in garlic portrayed positive impact in overcoming wilt caused by *Fusarium* while at the same time increased the plant growth by 30% to 40% (Sugiharto, 2019).

Furthermore, some species of *Penicillium* and *Paecilomyces* had been explored as potential bioremediator. For instance, *Penicillium citrinum* isolated from mangrove soil could absorb heavy metals notably lead, Pb (II) in an optimum condition where pH is 7, temperature of 30˚C and 60 minutes contact time (Wahab et al., 2017). A study also supported the same result but using different species; *Penicillium janthinellum* and *Penicillium brasilianum* which removed 91.2% and 74.4% lead respectively (Martins et al., 2016). In addition, a most recent research revealed the potential of *Penicillium piscarium* an important alternative for treating water contaminated with uranium. This fungus was able to remove between 93.2% to 97.5% uranium from solution contaminated with of 1 to 100 mg L^{-1} of uranium nitrate at pH 3.5, increasing the solution's pH to levels above 5.6 (Coelho et al., 2020). As for *Paecilomyces* sp., *P. formosus* could accumulate nickel, Ni in the mycelia without affecting its growth rate. This resulted in lower intake of Ni by soybean that was inoculated with the fungi and therefore alleviates the stress induced by Ni toxicity (Bilal et al., 2017). In addition, two *Paecilomyces* strain isolated from industrial dumping area could tolerate up to 6000 mg L^{-1} of cadmium, Cd through bioaccumulation of the heavy metal (Woldeamanuale, 2017), thus is beneficial in water treatment systems.

5. Conclusion

In conclusion, 10 fungi isolate from 4 different genera were successfully isolated from the used agricultural soil in Glasshouse and Nursery Complex, IIUM. The results showed significant variants in morphological characteristics thus it is speculated that the used agricultural soil harbors diverse fungal populations. Besides, in reference to previous studies, the identified genus of *Cladorrhinum, Penicillium, Paecilomyces* and *Aspergillus* sp. exhibit many potential applications that can be explored in various industries, not only limited to agriculture. However, further studies such as molecular identification is important in identifying and classifying soil fungi.

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