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The importance of culture-based techniques in the genomic era for assessing the taxonomy and diversity of soil fungi

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Abstract

Fungi are a diverse and highly abundant group of organisms found in soils worldwide. Understanding fungi is essential as they are key drivers of below-ground ecosystem functions. Taxonomy is a fundamental discipline, acting as the initial step toward biodiversity, ecology, and biotechnology studies. Both culture-dependent and -independent methods are employed in the taxonomic investigations of soil-dwelling taxa. High-throughput sequencing (HTS) is a genomic-based method widely applied in global studies that has revealed numerous unculturable soil taxa. However, this method is limited by its inability to link physical specimens to species identification. Culturing methods result in specimens that can be used to obtain genetic sequences and morphological data in applied studies. Thus, combining both methods is an important trend in taxonomic studies. This review discusses how culturing is important for soil fungal discovery and describes the main culturing methods. It also briefly addresses the role of HTS in taxonomy and its drawbacks, and the potential to combine both culture-dependent and -independent methods to gain better insights into soil fungi.

Keywords – Culturing methods – fungal taxonomy – fungi – high throughput sequencing – soil

Introduction

Fungi are one of the most abundant microeukaryotes with a cosmopolitan distribution. Their total number of species is a prolonged debate, and numerous investigations have continuously outnumbered the estimations. The latest estimates predict up to 12 (11.7–13.2) million species (Wu et al. 2019, Hyde et al. 2020c, Liimatainen et al. 2020, Zhang et al. 2021, He et al. 2022, Senanayake et al. 2022, Wijayawardene et al. 2022). Below-ground ecosystems, one gram of soil can contain between 5,000–10,000 microorganisms (Bridge & Spooner 2001, Van Elsas et al. 2019, Wagg et al. 2019, Tedersoo et al. 2020), and among these, fungi are one of the most prominent and

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diversified groups (Bills et al. 2004, Tedersoo et al. 2021, 2022). They have an essential ecological role as decomposers, mutualists, plant and animal pathogens, in nutrient cycling, and contributing to the mineral nutrition of plants and supply of carbon to other organisms (Hodkinson & Wookey 1999, Tedersoo et al. 2014, Jacoby et al. 2017, Averill et al. 2021, Bahram & Netherway 2022, Bhunjun et al. 2022). The superficial layers (up to 10–20 cm depth) "O" and/or "A" horizons contain suitable edaphic conditions for most fungi to develop due to the many organic resources and greater levels of moisture and aeration as compared to the deeper layers. Therefore, communities are generally higher in the surface layers (Schlatter et al. 2018, Peršoh et al. 2018).

Fungal taxonomy in soils is becoming an emergent field worldwide (Tedersoo et al. 2014, 2021, Wu et al. 2019). The Global Soil Mycobiome consortium (GSMc) reported that fungi represent 62.4% of OTUs (Operational Taxonomic Units), followed by Alveolata (15.1%), Metazoa (6.5%), Rhizaria (3.0%), and Viridiplantae (2.7%) (Tedersoo et al. 2021). Of the rich diversity mentioned in Tederso et al. (2021), 722,682 OTUs were recovered, comprising 45.7% *Ascomycota*, 28.3% *Basidiomycota*, 5.1% *Rozellomycota*, 3.1% *Glomeromycota*, and 2.7% *Chitridiomycota*. Currently, only around 800,000 world soil-inhabiting fungi are identified at the species level (Senanayake et al. 2022). Forest soils have the highest, while mud, riverbank, seashores, and polluted soils have a relatively lower diversity richness (Fracetto et al. 2013, Frac et al. 2018, Satyanarayana et al. 2019).

A holistic understanding of microbial diversity and taxonomy relies on knowing the functions of the taxa involved and how they interact with the ecosystem (Biswas & Sarkar 2018, Maharachchikumbura et al. 2021). Thus, the correct naming will help link the versatile knowledge of taxa worldwide. Due to technological and methodological limitations in sampling and resolution of detection techniques, the awareness of belowground fungi is limited, and integrating a reliable and effective method for assessing their taxonomy and diversity is an emergent challenge. Each method contains advantages and disadvantages (Hibbett et al. 2011, Orgiazzi et al. 2016, Cameron et al. 2018, Hongsanan et al. 2018, Wu et al. 2019, Semenov 2021). Conventionally, a polyphasic approach based on morphology, physiology, biochemistry, or reactions to chemical tests has been used to distinguish species (Senanayake et al. 2020). Currently, soil fungal research is based on culture-dependent and culture-independent methods (Tedersoo et al. 2017, Dissanayake et al. 2018, Jayawardena et al. 2018, Wu et al. 2019, Wijayawardene et al. 2021). The culture-independent methods use genomic technology to generate, analyse and interpret data from environmental samples. These data are extracted and analyzed using metagenetic, metagenomics (DNA-based), and metatranscriptomics (RNA-based) approaches (Gutleben et al. 2018, Semenov 2021). Metagenomics is used for functional profiling, and metagenetic is used for taxonomic community profiling using high-throughput sequencing (HTS) technology. High-throughput sequencing features next-generation sequencing (NGS) and third-generation sequencing (TGS) methodologies to determine fungal DNA data (O'Brien et al. 2005, Carvalhais et al. 2012, Schmidt et al. 2013, Raja et al. 2017, Sudhakar et al. 2018, Wu et al. 2019). This has spurred rapid advancements in recent years and is used for obtaining whole-genome sequences, delineating higher taxonomic levels, identifying closely related fungal species, describing the evolution of chemical gene clusters, and prioritizing industrially productive strains for bioactive compounds (among taxonomically related strains) discovery (Schmidt et al. 2013, Tedersoo et al. 2014, 2017, 2020, 2021, Raja et al. 2017, Sudhakar et al. 2018, Yang et al. 2019, Wu et al. 2019, Semenov 2021). From a taxonomic perspective, HTS is effective for revealing the unculturable soil taxa. However, these methods are still limited in classifying fungi to family or genus level (Tedersoo et al. 2017, Frac et al. 2018, Vu et al. 2019, Jurburg et al. 2021) as identification based on OTUs with short reads that lack a physical specimen cannot presently be used to accurately identify novel species (Tedersoo et al. 2017, Nagler et al. 2018, 2021, Hongsanan et al. 2018, Jurburg et al. 2021).

Culture-based techniques are essential to understanding taxonomy at the species level (Chambergo & Valencia 2016, Gutleben et al. 2018, Wu et al. 2019, Senanayake et al. 2020). This is where morphological illustrations and multigene phylogenies are integrated through living and dried cultures (Jeewon & Hyde 2007, Hongsanan et al. 2018, Chethana et al. 2020, Hyde et al.

2020c, Aime et al. 2021). Protocols to isolate and analyse soil-living species are found in numerous publications. Few recent soil studies have combined culture-based methods with HTS methods (Stefani et al. 2015, Selbmann et al. 2021). Different researchers have also used various methods, making it problematic to compare the results. For example, studies from 20–30 years ago have limited taxonomic information, and HTS methods have made information readily available and pertinent to initiate fungal culturing (Gutleben et al. 2018, Wu et al. 2019, Hyde et al. 2020a, b). Thus, the collection and standardization and freely available publication of protocols are needed. At the same time, it is also necessary to establish universal standards for the collection, transportation, and storage of soil samples (Gutleben et al. 2018, Wu et al. 2019, Senanayake et al. 2020). This is particularly important because a great deal of current taxonomic information has been obtained, which helps study the characteristics of miscellaneous soil fungal taxa and culture them (Wu et al. 2019, Hyde et al. 2020a, b).

This review addresses the importance of soil fungal-culturing methods in the genomic era and provides an account of existing culturing methods for studying soil fungal diversity, taxonomy, and other applications. We also discuss the status of investigative methods for soil fungi, including modern genomics methods in fungal taxonomy and their pros and cons. The value of fungal isolation methods and considerations in terms of modern soil fungal taxonomy is also addressed.

Importance of isolating and culturing fungi in modern taxonomy

Cultures (living or dry) of fungi provide physical samples that can be linked to the physiology and ecology of a species (Collado et al. 2007, Jeewon & Hyde 2007, Tedersoo et al. 2017, Aime et al. 2021). Unlike environment sequences, cultures can be stored, reproduced, and verified by molecular sequencing. Cultures are valuable for linking sexual and asexual morphs, and understanding functional guilds, and can be preserved as isolates (as dry and living) in reference collections. Cultures are also essential for screening biological activities, elucidating structures for biosynthesis and obtaining successful inoculants (Mueller et al. 2004, Guimarães et al. 2006, Kaewchai et al. 2009, Dorhout et al. 2011, Qiu et al. 2012, Rao et al. 2013, Knudsen & Dandurand 2014, Chambergo & Valencia 2016, Sena et al. 2018, Ramesh et al. 2019, Gurusinghe et al. 2019, Satyanarayana et al. 2019, Hyde et al. 2020b). In addition, due to the lack of cultivation, it has been predicted that 80% of fungal taxa in the soil are unidentified at the species level, and 20% cannot be assigned to an order (Tedersoo et al. 2017, Libor et al. 2019, Chethana et al. 2020, Senanayake et al. 2022).

Isolation and culturing methods for fungi

The employment of culture-dependent methods involves a series of steps, which can be summarized as follows: (i) a pure colony is isolated; (ii) the isolate is characterized by morphological and phylogenetic analysis; and (iii) morpho-molecular evidence is combined, providing to identify the species (Hawksworth & Rossman 1997, Stefani et al. 2015, Wu et al. 2019, Chethana et al. 2020, Aime et al. 2021). Growth media is essential as morphological characteristics can be produced *in vitro* (Hawksworth & Rossman 1997, Stefani et al. 2015). The morphology is based on macroscopic characteristics (form, size, colour, and growth rates of cultures) and microscopic characteristics of reproductive structures of asexual and/or sexual morphs (Jeewon & Hyde 2007, Rosas-Medina et al. 2019). Cultures help observe and describe specific structures not present in natural states, such as synasexual morphs, different conidia (alpha, beta, or gamma), and chlamydospores which help document the taxa (Domsch et al.1993, Wijayawardene et al. 2021). Morphology is illustrated through images, drawings, and descriptions (Raja et al. 2017, Rosas-Medina et al. 2019, Senanayake et al. 2020).

Some taxa (*Xylariales* and macrofungi) produce fruiting bodies on plant litter or the surface of the earth that are easy to observe. Fast-growing strains can also easily be isolated and grown in artificial media (Jeewon & Hyde 2007). However, many species are present in soils without forming visible phenotypic characteristics, dwell in uncommon or extreme conditions, and some are remarkedly slow-growing (Bridge & Spooner 2001, Stefani et al. 2015, Senanayake et al.

2022). Furthermore, some taxa are exclusively adapted to their substrates; hence, the *in-vitro* conditions of isolation limit their growth. Therefore, selecting isolation methods is critical for isolation success (Bridge & Spooner 2001). Inducing *in vitro* conditions similar to original edaphic conditions enhances the isolation frequency and growth (Grossbard 1952, Kazerooni et al. 2017).

Recovering unculturable species is a major challenge in culture-dependent methods as artificial media can inhibit growth (Grossbard 1952, Kazerooni et al. 2017). For example, symbiotic relationships cannot be maintained in vitro, precluding the growth of these organisms (Jeewon & Hyde 2007, Kazerooni et al. 2017). Besides, fast-growing species overgrow slow-growing (Jeewon & Hyde 2007), and some release antibiotics, inhibiting the germination and growth of other strains (Grossbard 1952).

The following section provides an overview of currently available isolation techniques for investigating soil fungi (Table 1).

Table 1 Available method to isolate different fungal taxa from soil.

Fungal isolation method	Isolated fungal group	References
Baiting method	*Ascomycota* and basal fungi	Butler (1907), Simpanya & Baxter (1996), Giudice et al. (2012), Abu-Mejdad (2013), Altayyar et al. (2016), Masoudi et al. (2020), Senanayake et al. (2020)
Dilution plate method	*Ascomycota*, *Basidiomycota*, and basal fungi	Waksman (1922), Brierley et al. (1928), Kutateladze et al. (2016), Das et al. (2019, 2020), Park et al. (2020), Santos et al. (2020), Senanayake et al. (2020), Yasanthika et al. (2020)
Soil plate method	*Ascomycota*	Warcup (1950), Parkinson & Thomas (1965), Kazerooni et al. (2017), Senanayake et al. (2020)
Chemical pasteurization (alcohol and acid treatment methods)	*Coprophilous*, *Ascomycota*, ascosporic, and sclerotia producing *Eurotiales* and cleistothecial and perithecial *Ascomycota*	Kuehn & Orr (1962), Warcup & Baker (1963), Bills et al. (2004)
Heat treatment method	*Ascomycota*	Warcup & Baker (1963), Ali et al. (2009)
Soil steaming method	*Ascomycota*	Warcup et al. (1951)
Soil immersion tube	*Ascomycota*	Chesters (1948), Mueller & Durrell (1957), Garrett (1981), Mukerji (2002), Senanayake et al. (2020)
Isolation of fungal hyphae attached to soil particles	*Ascomycota*	Warcup (1955)
Soil washing technique	*Mycorrhizal*, *Ascomycota* *Mucorales*, and *Basidiomycetes*	Parkinson & Williams (1960), Azaz (2003), Pacioni (1991)
Wet sieving and decanting method	Mycorrhizal fungi and endogenous fungi	Gerdemann & Nicolson (1963)
The Mankau method for isolation of nematophagous fungi in soil	*Ascomycota*	Mankau (1975)
Fungal highway columns method	Arbuscular mycorrhizal fungi	Simon et al. (2015)

Baiting method

Baiting, initiated by Butler (1907), is one of the most effective methods for isolating soil fungi (Meyling et al. 2007). The method aids in isolating substrate-specific taxa (cellulophillic fungi, keratinophilic fungi, and entomopathogenic fungi) and establishing their trophic nature (Mueller et al. 2004, Giudice et al. 2012, Abu-Mejdad 2013). In this method, sterilized organic

substances are placed in soil samples and incubated. The growth of fungi on the substrates is observed, and propagules are transferred to sterilized nutrient media.

Different baits provide various nutrient sources, and therefore it is possible to isolate a wide range of fungal taxa (Meyling et al. 2007). Table 2 lists the various organic substrates that were used in previous studies. To examine the biocontrol activity of taxa against target hosts, weed leaves, other fungi, insects, and nematodes can be used as baits (Meyling et al. 2007). For example, *Galleria mellonella* and *Tenebrio molitor* larvae are commonly used to isolate entomopathogenic fungi from soil (Tuininga et al. 2009, Sharma et al. 2018). For this, larvae are placed in a sealed plastic container filled with a soil sample and the resulting larvae carcass is dissected and placed on culture media to observe mycelial growth (Tuininga et al. 2009, Sharma et al. 2018).

Furthermore, the baiting method have successfully been used as a microcosm to recover real-time interactions of fungi and bacteria under natural soil conditions (Ballhausen et al. 2015, Ghodsalavi et al. 2017). Bait can be treated with an antibiotic solution (20% Clorox, 0.2 g/mL chloramphenicol -or strepto-penicillin at 1000 IU/mL) to avoid bacterial contamination (Manoch 1998, Pakshir et al. 2013). The general steps involved in the baiting method for isolating soil fungi are given in Fig. 1.

Table 2 Baits used to isolate fungi and species recovered.

Baits	**Fungal genera/groups**	**Incubation period**	**References**
Hair	*Mycrosporum* (Kerotinophillic fungi)	Poultry farm soil for 3 weeks	Shukia et al. (2003)
Hair	*Aspergillus*, *Acremonium*, *Chrysosporium*, *Microsporum*, *Trichoderma*	3–4 weeks	Altayyar et al. (2016)
Corn leaf, snake skin, pine pollen, and hemp seed	*Catenaria*, *Rhizoptrydiums*	ND	Sundaram (1977)
Human hair and Fowl's feather wool	*Aspergillus*, *Chrysosporium*, *Microsporum*	ND	Sundaram (1977)
Leaves and ticks	*Beauveria, Metarhizium*	ND	Tuininga et al. (2009)
Tenebrio molitor (meal worm beetle)	*Beauveria*, *Cordyceps*, *Purpureocillium*	ND	Sharma et al. (2018)
Vegetable pieces, rice, and pine	89 fungal genera		Manoch (1998
Apple, black pepper leaves, black pepper leave discs, and castor seeds	*Phytophthora*	ND	Holliday & Mowat (1963), Kueh & Khew (1982), Ramachandran et al. (1986), Sastry & Hegde (1988)
Sterilized wood pieces (at least 2 cm^2)	*Ramophialophora* sp.	ND	Calduch et al. (2004)
Hair	*Chrysosporium* sp.	ND	Călin et al. (2017)
Albizia falcafaria leaflets	*Phytophthora*	ND	Anandaraj & Sarma (1990)
Goat hair	*Alternaria, Aspergillus, Candelabrella*, *Chaetomium, Chaetomium, Cladosporium, Cunninghamella, Drechslera, Emericella*, *Eurotium, Fusarium, Fonsecaea, Geotrichum*, *Geotricum, Graphium*, *Gliocladium, Giodianichiella, Haplosporangium, Helmithosporium,*	14 days on sterilized Petri dishes	Ramadan & Ismael (2011)

Table 2 Continued.

Baits	Fungal genera/ groups	Incubation period	References
	Macrophomina, *Monocillium*, *Neosartoria*, *Paecilomyces*, *Penicillium*, *Phoma*, *Pythium*, *Scytalidium*, *Stemphylium*, *Sclerotium*, *Syncephalastrum*, *Tetracoccosporium*, *Thermoascus*, *Tricladium*, *Trichosporium*, *Verticillium*, *Rhizomucor*, and Yeasts		

(ND: Not determined)

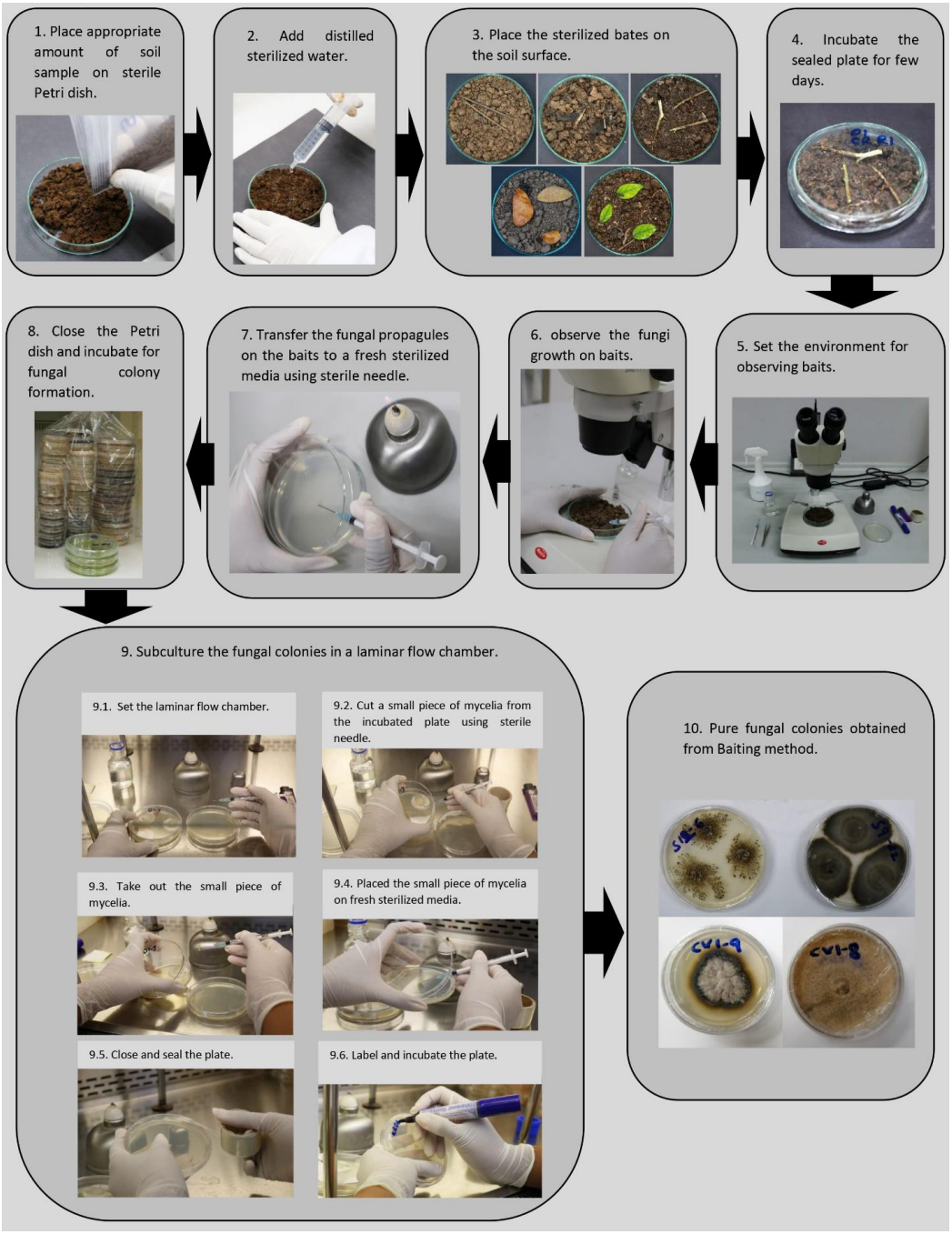

Figure 1 – Baiting technique for the isolation of soil fungi.

Dilution plate method

Different soil samples might contain miscellaneous densities of fungal propagules. Low densities of colonies in culture media minimize inter-colony interactions, favouring obtaining pure fungal strains (Bills et al. 2004). The soil: water ratio is adjusted to obtain several colonies (5–30) in a 100 mm Petri-dish plate in the dilution plate method. Waksman (1922) introduced the dilution plate technique for soil bacteria isolation, and Brierley et al. (1928) modified it for fungal isolations from the soil. Most of these taxa are saprobes and can withstand microbial antagonism (Ramakrishnan 1955). This technique is widely used for quantitative and qualitative estimations of the species (e.g., Aziz & Zainol 2018, Marin-Felix et al. 2019, Alotaibi et al. 2020, Alidadi et al. 2020, Guevara-Suarez et al. 2020). The steps of the method vary according to the study objectives (Aghamirian & Ghiasian 2013, Aziz & Zainol 2018). The basic steps of the dilution method are illustrated in Fig. 2.

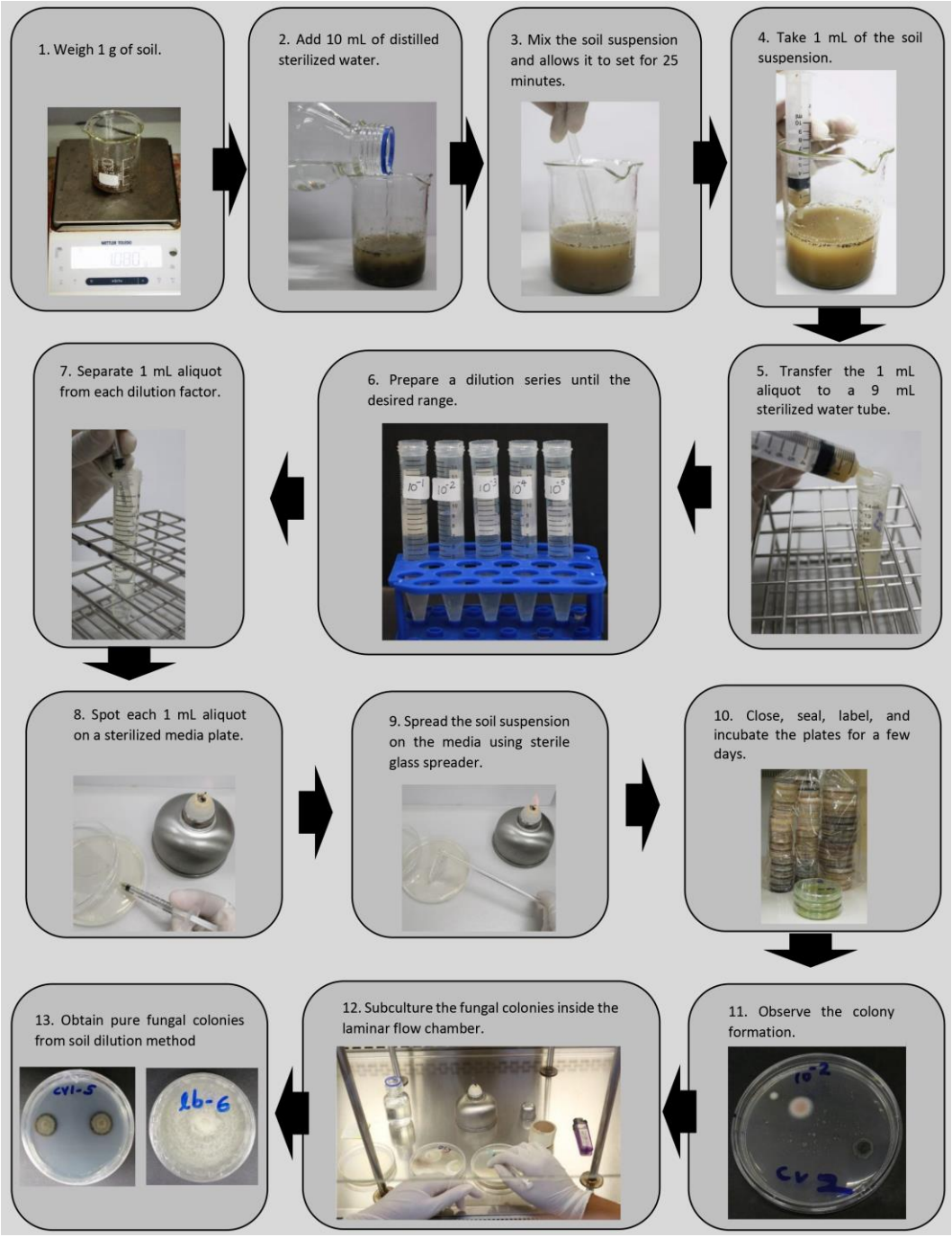

Figure 2 – Dilution method for the isolation of soil fungi.

Soil plate method

The plating method for isolating soil-inhabiting species was introduced by Warcup (1950). In this method, the soil is placed directly beneath the growth media to obtain cultures. Soil samples are weighed to 0.015 g and placed in the sterile Petri-dish, and a thin layer of sterile warm melted Czapek-Dox + yeast extract, pH 5.0 agar, or glucose ammonium nitrate (GAN) media is poured over the soil layer. Plates are incubated at room temperature, preferably for 3–5 days, for colony growth (Warcup 1950, Manoch 1998). In their experiments, the authors obtained hyphomycetes, *Pythium*, and *Mortierella* species. This method was successfully used, with minor modifications, by Parkinson & Thomas (1965) to isolate rhizosphere fungi. The steps for utilizing the soil plate method are illustrated in Fig. 3.

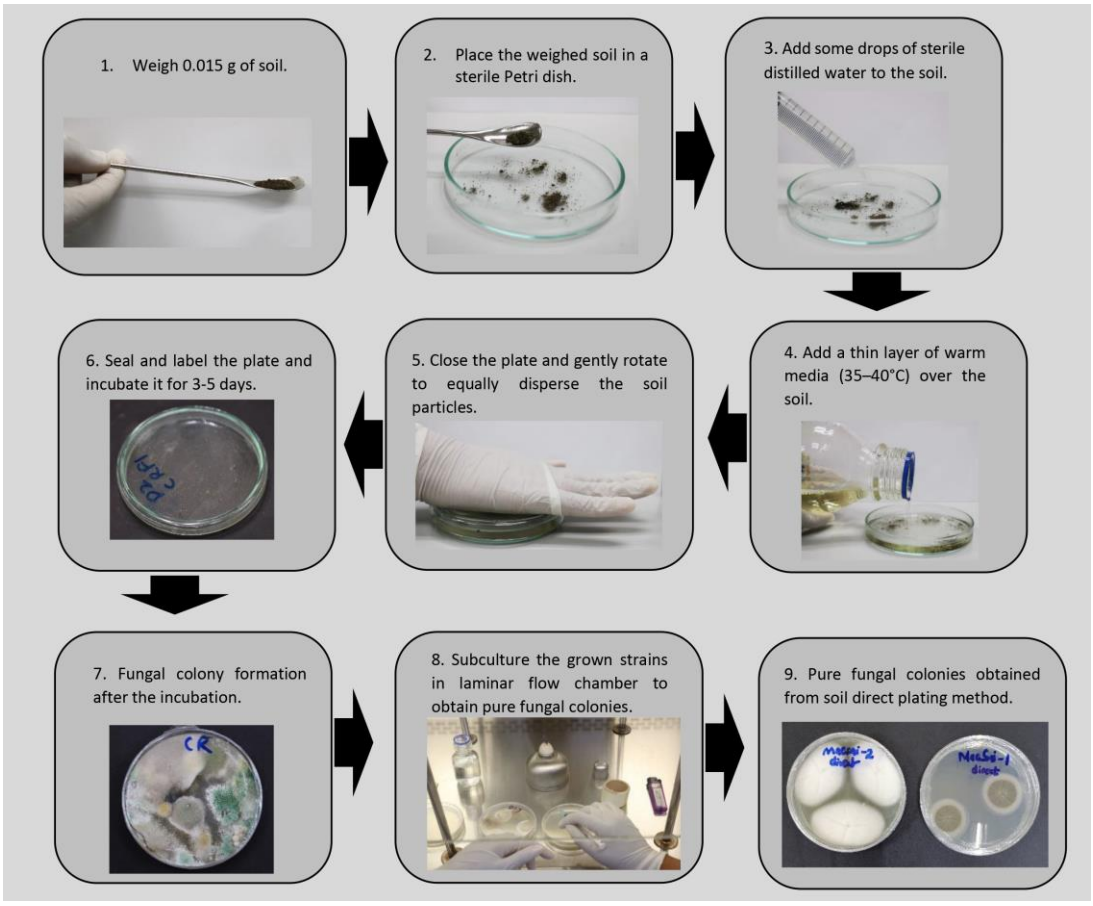

Figure 3 – Soil plating method for the isolation of soil fungi.

Alcohol treatment method (Chemical pasteurization)

This method was first described by Warcup & Baker (1963). It consists of weighing approximately 0.3 g of soil, placing it in an 18 mm sterilized dry test tube, and adding 65–70% ethanol. After that, the solution is shaken thoroughly and left for 10–15 minutes to settle. The excess alcohol is removed, and the soil is transferred to the sterile Petri-dish using a spatula. A thin layer of warmed GAN media at 45 °C is then poured over the soil layer. Petri dishes are shaken gently until soil particles are distributed well across the bottom. Plates are incubated for 3–5 days at 26–28 °C (Fig. 4). Once the hyphal growth is obtained, its tips are transferred into the potato dextrose agar (PDA) slants and kept at 26–28 °C for two weeks to allow culture growth (Warcup & Baker 1963, Abdullah & Saadullah 2013).

Acid treatment (Chemical pasteurization)

In a study by Kuehn & Orr (1962), acid was added in 0.00015, 0.0005, and 0.00075 mg/mL into melted PDA and poured onto the Petri-dish. A small amount of soil was added to the media

and incubated for 3–5 days. After obtaining the hyphal growth, the hyphal tips were transferred to PDA slants using a sterile needle. Finally, PDA plates were incubated at 26–28 °C for two weeks until fungal colonies were obtained. The method was later adapted by Furuya & Naito (1979), in which 3 g of soil were weighed and mixed with 5% acetic acid in a glass tube. The mixture settles for 20 minutes, and the surface acid layer is discarded. Next, the soil is washed with 10 mL of sterilized distilled water, and 1 mL of soil suspension is poured over the sterilized malt-extract agar (MEA) media (Fig. 5).

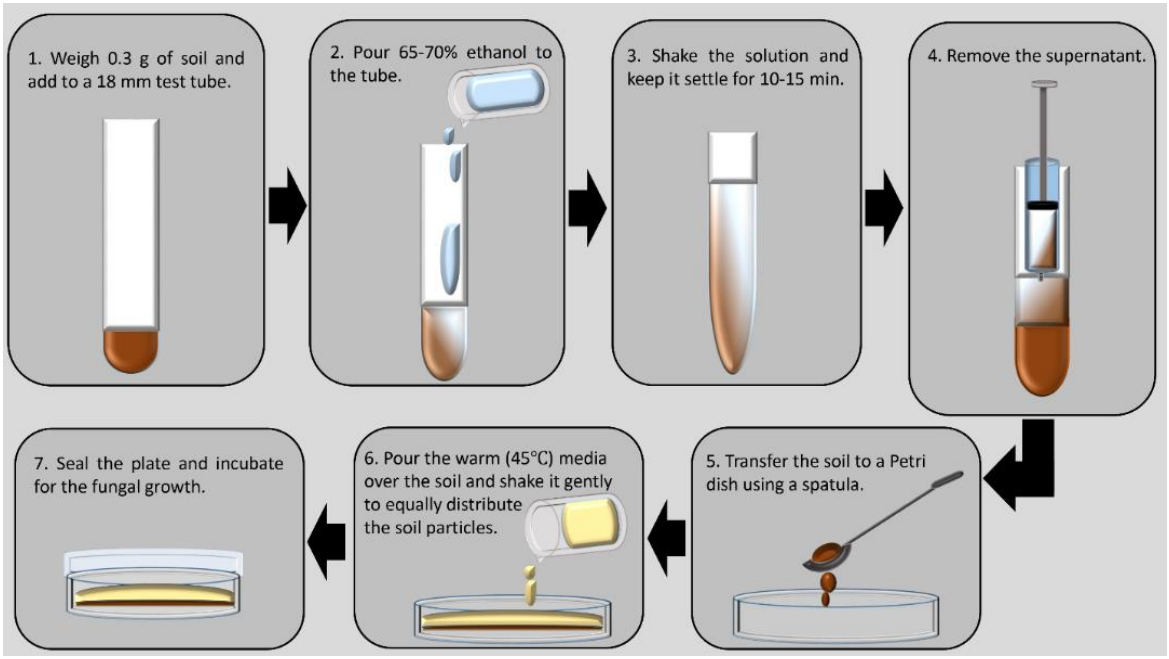

Figure 4 – Alcohol treatment method for the isolation of soil fungi.

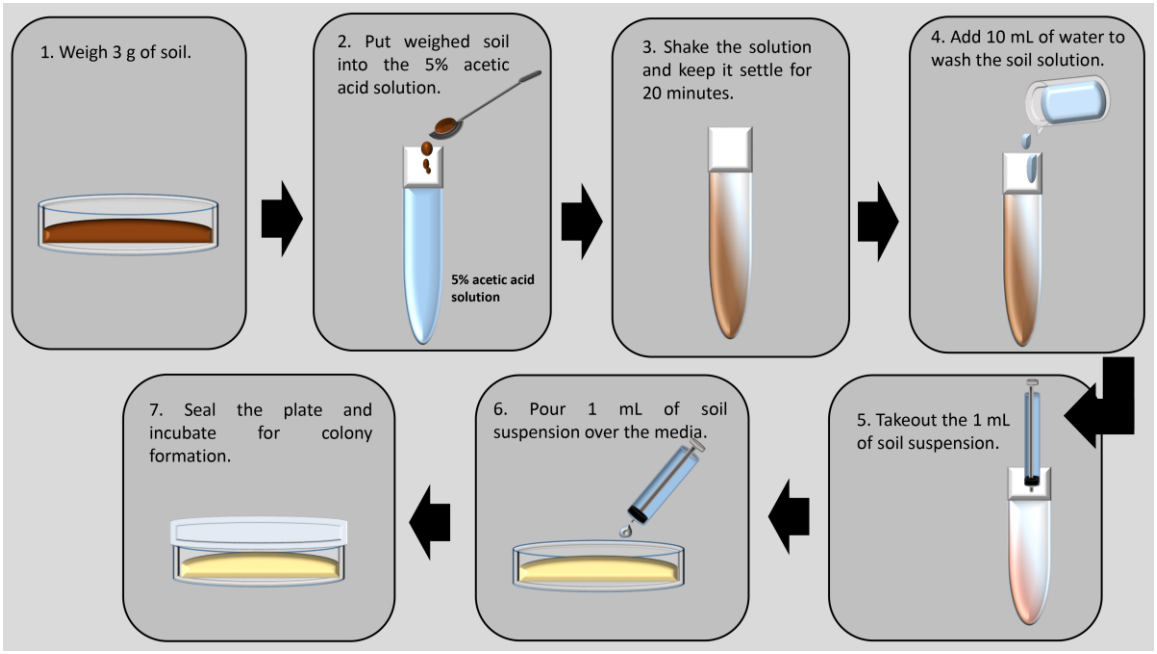

Figure 5 – The acid treatment method for the isolation of soil fungi.

Heat treatment method

Warcup & Baker (1963) introduced the heat treatment method to isolate fungi from the soil. Many heat-resistant ascomycetes demand heat activation before germination of ascospores (Katan

1985, Ali et al. 2009); thus, this method can isolate heat-resistant strains (Ali et al. 2009). Heat inactivates the vegetative parts and less heat-resistant sexual spores of fungi but suppresses the activities of bacteria (Beuchat & Pitt 1992). In this method, 0.3 g of soil is placed in a sterile 18 mm test tube, and sterile distilled water is added. The soil solution is kept in a water bath at 60–80 °C for 10–15 minutes. Once the soil settles, excess water is removed, and the soil is placed in a sterile Petri dish. Glucose ammonium nitrate media is poured over the soil layer and gently rotated to distribute the soil in the bottom of the Petri-dish (Fig. 6). The Petri dishes are then incubated for 30 days between 26–30 °C. The colonization begins after the fifth day, maturing after 14 days. Hence, observation of colony growth generally occurs between days 7 and 14. Once the grown hyphae are observed, their tips are transferred to the new PDA medium to obtain isolates (Warcup & Baker 1963, Abdullah & Saadullah 2013).

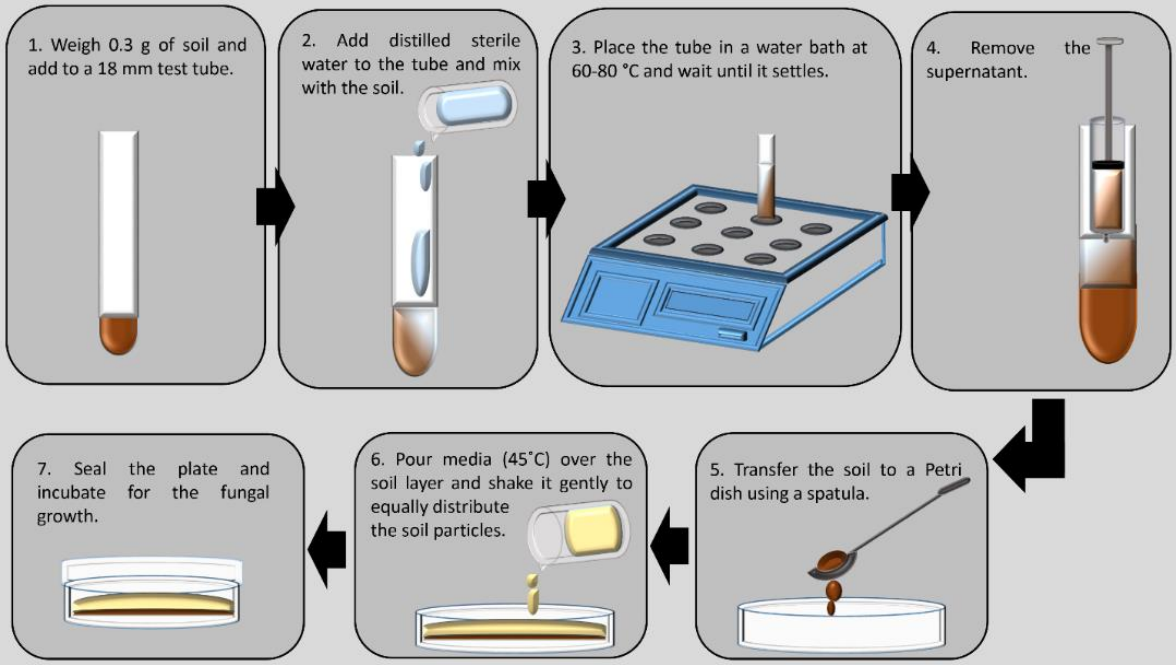

Figure 6 – The heat treatment method for the isolation of soil fungi.

Soil immersion tube

This method was first used to isolate other microorganisms from soils (Chesters et al. 1940, Chester et al. 1948) and has been characterized as highly selective for isolating species that can tolerate low oxygen levels (Chesters & Thornton 1956). The method was later modified by Gochenaur (1964) and several other authors to reduce selectivity and permit the isolation of a broader range of species (Thornton 1952, Parkinson & Kendrick 1960). A tube with 4–6 spirally arranged mini-holes containing nutrient agar is placed in the soil, separated by an air gap. After 5–8 days, the tubes are brought to the laboratory, where the agar core is taken out and cut into pieces. Pieces with mycelia are placed in sterilized media, and subsequently, colonies can be sub-cultured in selective media (Mueller & Durrell 1957, Mukerji 2002). The basic steps of the method are illustrated in Fig. 7. When using the original method, the core of the agar tube contains less aeration, which makes it suitable for isolating low oxygen-tolerant, fast-growing fungi. This method also isolates non-sporulating fungi such as *Rhizoctonia solani* (Chester et al. 1940, 1948, Garrett 1981).

Soil steaming method

Soil steaming is a selective method for isolating slow-growing ascomycetes and hyphomycetes from the soil (Warcup et al. 1951) that are likely to be overgrown by fast-growing groups such as *Absidia*, *Mortierella*, *Mucor*, *Trichoderma*, and *Zygorhynchus*. The method consists

of weighing 125 g of soil into a glass tumbler and steaming it for 2, 4, 6, 8, and 10 minutes at 100 °C. One cm from the surface soil layer in each tumbler is removed and transferred separately to 10 Petri-dishes containing Dox+yeast extract agar media (acidified with phosphoric acid to pH 4). This is then steamed for four minutes. The most efficient duration is four minutes to reduce fast-growing taxa, and ascomycetes resistant to a short steam treatment can also be isolated. This is favoured by the small number of colonies grown on the plates (Warcup et al. 1951). Heavy soils or soils with a high proportion of humus may require more extended steaming periods. However, over-steaming can inactive colony formation (Warcup et al. 1951).

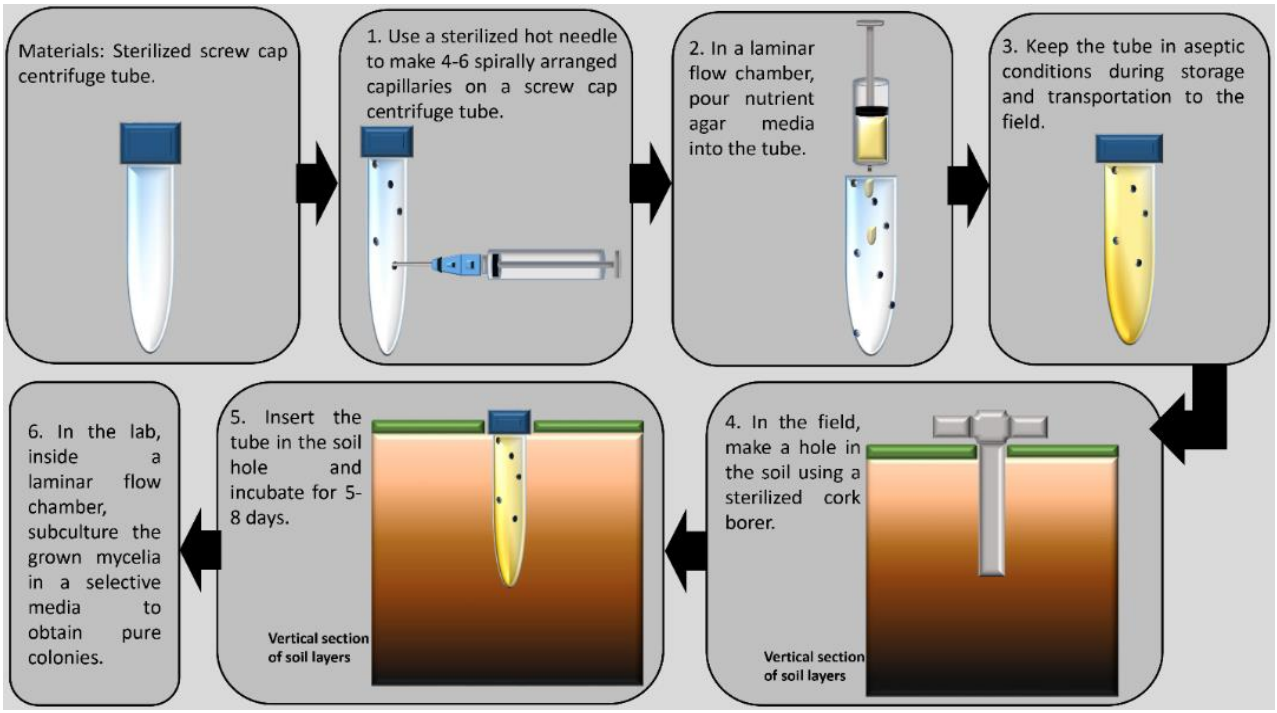

Figure 7 – Chester's soil immersion tube method for the isolation of soil fungi.

Isolation of fungal hyphae attached to soil particles

Most fungal mycelia in soil are attached to larger particles, and removing fine particles eases their observation and isolation from the soil (Warcup 1955). For the procedure, 1–1.5 g of soil is placed in sterilized water in a part-filled beaker for 4–5 minutes. After that, the crumbled soil particles are broken using a water jet, and the suspension is left to settle for 60–90 seconds. After sedimentation is completed, the excess suspension is removed, and the process is repeated several times until the suspension becomes clear. Finally, a small amount of water is added to the soil sedimentation to create a soil solution, and the solution is distributed across three sterile Petri-dishes. The presence of hyphae attached to the soil is observed using a stereomicroscope. The hyphal masses or parts of hyphae are removed using fine forceps. Then it is deposited on the sterile water drop placed on sterile Petri-dishes, and melted cooled agar is poured over the hyphal water solution. After incubation for a few days at room temperature, hyphae grown on media are transferred to a new sterilized Dox+agar media plate. Ammonium salt, peptone, soil extract, or hydrolysed casein yeast extract can also be added to the medium to stimulate hyphal growth (Warcup 1955).

Isolation of rhizosphere and mycorrhizal fungi

Wet sieving and decanting method

Plant nematologists commonly use wet sieving and decanting techniques to extract nematode cysts and larvae from the soil (Triffitt 1935). Gerdemann & Nicolson (1963) used this method to

obtain fungal *Endogone* spores from the soil. This technique consists of suspending 100 g of soil in litre of water, waiting some seconds for the heavier particles to settle, and sieving the suspension by passing it through a 1 mm sieve to remove larger particles of organic matter. The suspension that passes through is stirred to re-suspended. After the heavier particles settle for a few seconds, the decanted liquid is filtered in a sieve fine enough (1–40 µm) to retain the spores. Finally, retained content of all the sieves is transferred to a Petri-dish and examined with a stereoscopic microscope. The spores and sporocarps identified in the mixture are individually picked up using a flattened needle and placed in a small concave dish of water for further use (Gerdemann & Nicolson 1963, An et al. 1990, Srinath et al. 2003). Several modifications were introduced, such as the optimal ratio as 1:10 in the soil: water proportion, stirring the suspension by a magnet stir using a water-driven stirrer without heat for 10 minutes, the addition of 0.1–0.5% of Tween 80 and 0.1 M of sodium pyrophosphate if the soil is clay (Pacioni 1991).

The method to isolate nematophagous soil fungi

Mankau (1975) introduced this method to quantify soil-borne nematophagous fungal colonies. Ten grams of soil are weighed, sieved using a 2 mm mesh, soaked in distilled water for 10 minutes, and suspended in 500 mL of distilled water. The suspension is sieved through graded sieves of 500 µm, 125 µm, and 35 µm. The residue on each sieve is collected into a flask and allowed to stand briefly for the heavy mineral particles to settle, and the remaining suspension is discarded. The residual materials are vacuum-filtered on filter paper discs. The filter paper disks are removed, cut into halves, and each half is placed on a cornmeal agar or water agar plate to obtain colonies (Mankau 1975, Bailey & Gray 1989).

Novel techniques introduced to isolate soil fungi

Fungal highway columns method

Fungi and bacteria are involved in many soil interactions. Fungal highways are one such interaction in which bacteria disperse along fungal mycelium. These interactions are still poorly studied (Simon et al. 2015). Simon et al. (2015) introduced this method to identify fungal and bacterial species involved in fungal highway interactions. In the pilot study, Simon et al. (2015) isolated and verified the associations between *Chaetomium globosum* × *Acinetobacter* sp., *Fusarium oxysporum* × *Ochrobactrum* sp. and *Stenotrophomonas maltophilia*, and *F. equiseti* × *Achromobacter spanius*, and *Stenotrophomonas humi*.

The fungal highway column separates coexisting fungi and bacteria in their associations (Simon et al. 2015). The device contains columns with two agar plugs containing culture media (Fig. 8). The interior of the column is designed to be the nutritional habitat for both fungal and bacterial growth. Within the column, the culture media is separated into two compartments. Filamentous fungi are separately isolated into the top, and bacteria and some other fungi are separated into the underneath compartment (Fig. 8) (See Simon et al. [2015] for more details). The diversity of isolated fungi depends on the nutrient composition of the media in the column.

For using the fungal highway column, the bottom cap is removed, and the column is placed on the desired substrate and left for 4–10 days. After removing the substrate, the column is immediately closed with another sterilized bottom cap. Directly, or after 8–15 days of incubation, the cap on the top of the column is removed in a sterile environment, and the target culture medium is plated for the isolation of cultivable organisms or directly used for nucleic acids extraction (Simon et al. 2015). Junier et al. (2021) modified this method to isolate soil bacterial-fungal associations.

A brief comparison of different isolation methods

Each culture-dependent method consists of specific procedures and presents unique benefits (Kutateladze et al. 2016). Kumar et al. (2015) obtained higher species diversity and abundant sporulation in the dilution plate method over the soil plate method using the same soil samples.

Abdullah & Saadullah (2013) stated that the dilution plate method isolates more colonies than the alcohol treatment method. Several *Ascomycota* (*Apisordaria, Chaetomium, Gelasinospora, Neosartoria, Sordaria,* and *Talaromyces*) were obtained using heat and alcohol treatments (Manoch 1998). The selective isolation of soil *Ascomycota* can be done by heat and chemically pasteurized soils (Warcup 1951, Warcup & Baker 1963, Samson & Luiten 1975, Furuya & Naito 1979, 1980). Moreover, heat, ethanol, or other organic solvent treatments limit fungal isolations from thin-walled conidia or hyphal fragments and facilitate isolating strains from ascospores, sclerotia, and thick-walled conidia or hyphae (Furuya & Naito 1980). Furuya & Naito (1980) and Bills et al. (2001) suggested that pre-treating soil with heat or small organic molecules can facilitate ascospore germination in some taxa.

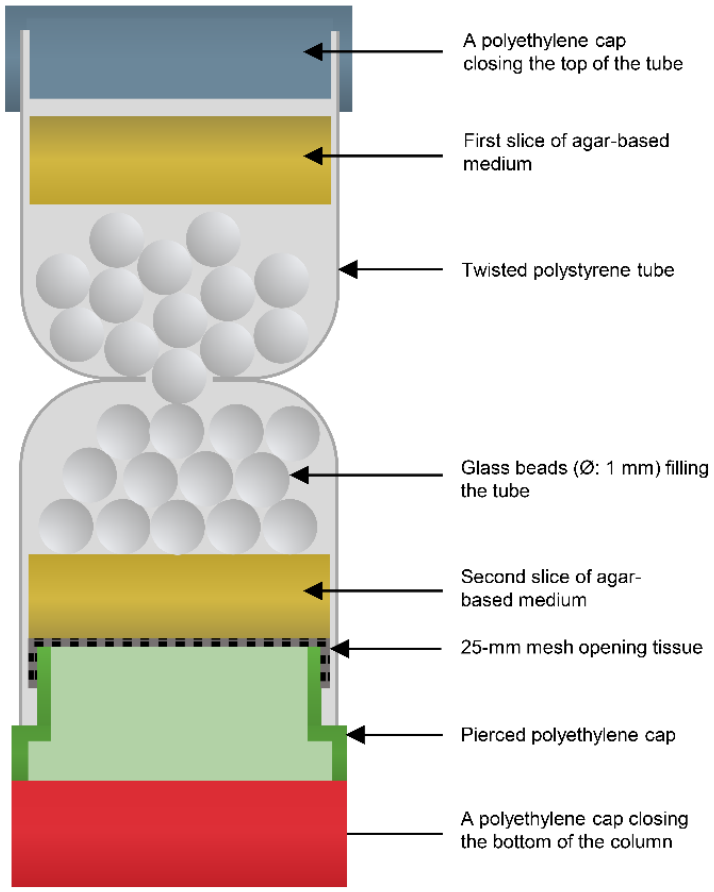

Figure 8 – Design and parts of the fungal highway column.

Sample collection, media selection, growth conditions, and preservation of cultures from soil studies

Soil sample collection

Sample collection is the first step in soil fungal studies. Generally, the A1 horizon is collected for biological diversity studies. Before collecting, litter or non-soil materials should be removed from the surface of the collection site. Depending on the purpose, tools such as a shovel, auger, knife, spatula, or spoon can be used (Mueller et al. 2004, Senanayake et al. 2020). The tool of choice and gloves must be sterilized with 70% ethanol and dried before use. Usually, 10–30g of soil is collected from each location; however, this depends on the study objectives. Samples can be separately analysed or homogenized to represent a large study area. Ziploc bags or sterilized bags can store the samples, which should be maintained at 0–10 °C during transportation and short-term storage. It is advisable to isolate fungi immediately from the fresh samples within a few days.

Otherwise, soil samples can be air-dried in sterile conditions (laminar flow hood can be used) at 25 °C and placed in cold storage or freezers. Temperate soil samples can be stored at -10 to -20°C to eliminate the effects of temporal changes (Mueller et al. 2004).

Media selection and growth conditions

The culture media is critical when growing fungi *in vitro*, mainly because the growth requirements (nutrients, pH, osmotic conditions, and temperature) and patterns differ among taxa (Basu et al. 2015). They can be present as mycelia, non-mycelial spores (chlamydospore), and sexual/asexual spores on the soil that can have different sensitivity to growth media (Tsao & Ocana 1969). As fungi are substrate-specific, previous knowledge of the sampling location (climate, soil type, soil temperature, and vegetation) is vital for selecting and preparing ideal media (Basu et al. 2015, Kumar et al. 2015).

Both natural media and synthetic media are used for isolation. Because of the low cost and ease of production, the natural media, corn meal agar (CMA), PDA, and dung agar are widely used. However, the nutritional composition range in natural media is undetermined, while synthetic media are produced using precise amounts of ingredients, finely controlled temperature, and pH conditions. However, media selection depends considerably on research objectives and resource availability (Bills et al. 2004, Kumar et al. 2015). Most soil studies use PDA, which is suitable for mycelia growth and facilitates a wide range of taxa (Kumar et al. 2015). Calduch et al. (2004) used potato carrot and oatmeal agar to grow propagules isolated from the baiting technique. Agar medium has been used as the isolation medium for coexisting fungi and bacteria in the fungal highways method (Simon et al. 2015). Manoch (1998) used GAN in the dilution plate method to obtain cultures, followed by the subculturing on PDA and Czapek-Dox agar medium to obtain *Aspergillus* and *Penicillium*. Warcup (1950) mentioned that Czapek-Dox + 0.5% yeast extract agar with Phosphoric acid (pH 5.0) is a suitable medium for sporulating and isolating soil fungi.

The pH of the media is critical for inducing the growth of certain taxa while inhibiting others (Chesters & Thornton 1956). For acidification, sulphur, citric, lactic, and phosphoric acids and alkalization NaOH are added to the medium (Warcup 1950, Azaz 2003, Kazerooni et al. 2017). Antibiotics (penicillin, streptomycin, chloramphenicol, chlortetracycline, oxytetracycline, tetracycline, kanamycin, pimaricin, and vancomycin) and antimicrobial chemicals (sodium propionate, rose Bengal, crystal violet, malachite green, gallic acid, pentachloronitrobenzene and phenols) are used to exclude and control for unwanted organisms in the media (Tsao 1970, Hunter et al. 1974). Fungicides or antifungal compounds can select specific taxa and help obtain pure fungal colonies (Tsao & Ocana 1969, Tsao 1970, Hunter et al. 1974, Bills et al. 2004). Tsao & Ocana (1969) mentioned that 100 p.p.m. (parts per million) pimaricin and 200 p.p.m. pentachloronitrobenzene could delay or prevent germinating non-pythiaceous taxa, and vancomycin 100 p.p.m. can suppress actinomycetes and bacterial growth in the media which can successfully isolate soil dwelling *Phytophthora* species. Dodine has been used to selectively isolate entomopathogenic hyphomycetes from soil, including some of Metarhizium and Beauveria (Fernandes et al. 2010). For isolation of entomopathogenic fungi, glucose, Oxgall, and peptone have been added to the media with antibiotics such as rose Bengal, chloramphenicol, and cycloheximide (Veen & Ferron 1966). *Trichoderma* selective medium was introduced by Elad et al. (1981), which was developed using chloramphenicol for bacterial inhibition and pentachloronitrobenzene, p-dimethylaminobenzenediazo sodium sulfonate, and rose Bengal for fungal inhibition.

Incubation conditions and storage environment of the isolates need to be set according to the thermo-sensitivity and the photo-sensitivity of the media and the antimicromicrobial/chemical substance (Tsao & Ocana 1969). Most polyene antibiotics are photosensitive and media containing those substances necessary to store/incubate under dark conditions.

Nevertheless, constraints are more critical when isolating and growing extremophiles because many media are unstable above 70 °C (Tsudome et al. 2009, Basu et al. 2015). In some successful cases, Durán et al. (2019) isolated psychrotrophic soil fungi that grow above 20 °C and

psychrophilic soil fungi that grow below 20 °C. Carreiro & Koske (1992) used different temperature conditions (0 and 25 °C) for isolating extremophiles. At 0 °C, many psychrotrophic and some psychrophiles (e.g., *Mortierella* and *Mucor* sp.) were isolated, while mesophiles were isolated at 25 °C. Satyanarayana et al. (2019) isolated thermophilic soil fungi at 41–122 °C. A porous solid plate made of nano-fibrous cellulose media can be stable at 260 °C in 25 MPa, which has the potential for *in vitro* fungi isolation from extreme soils (Tsudome et al. 2009, Basu et al. 2015).

Preservation of soil fungal cultures

Fungal strains can be preserved as fresh materials, dried cultures, and permanent slides (Mueller et al. 2004). Dried cultures and permanent microscope slides are stored at fungaria (Mueller et al. 2004, Senanayake et al. 2020). These preserved strains are labelled with specific numbers with taxonomic and biogeographical data and other metadata such as host, substrate, collector's name, and collected date (Dentinger et al. 2016).

For the preparation of dried cultures, the fungal strains are grown in a water agar medium (2.5% glycerol). Properly labelled strains are subjected to warm air drying or drying in a desiccator. Dried cultures can be stored in wax paper bags with silica gel, paper envelopes, or thin Petri dishes for deposit in the herbarium (Mueller et al. 2004, Silva et al. 2011). These materials can be used for DNA extractions and later morphological studies (Senanayake et al. 2020).

To preserve fungal structures, tissues containing them are mounted on microscopic slides, which can be reused for a long time. This method is beneficial for preserving fungal-type specimens with rare or important morphological structures (Foster et al. 2011). Kohlmeyer & Kohlmeyer (1972) stated that the steps involved in this technique are fixation, dehydrating, clearing, and mounting. For fixation, 70% alcohol, Bouin's fluid, or formalin are used, and glycerin can be used as the mounting agent finally, the slide is sealed using a coverslip and colorless nail polish (Kohlmeyer & Kohlmeyer 1972, Senanayaka et al. 2020). Fresh culture preservations can be submerged in 70% ethanol, Kew mixture, or Copenhagen solution with glycerol to preserve their living phase (Mueller et al. 2004).

The value and potential of culturing soil fungi

There are significant benefits stemming from culture-dependent methods (Jeewon & Hyde 2007, Rao et al. 2013). Genomic DNA extractions from pure cultures, sequence data, and phylogenetic analyses enhance the identification accuracy. Currently, DNA extraction from cultures is done by commercial DNA extraction kits and CTAB methodology (Cetyl trimethylammonium bromide) (Rao et al. 2013, Dissanayake et al. 2020, Wijayawardene et al. 2021). Thus, cultures represent a source of fungal DNA and help to preserve the DNA of the species (Rao et al. 2013, Rincon-Florez et al. 2013, Wu et al. 2019, Chethana et al. 2020, Wijayawardene et al. 2021). Some culture methods, such as baiting and the fungal highway column method, help study the nutritional modes inherent among soil groups. Using different growth media and *in vitro* conditions is beneficial for detecting slow-growing taxa and observing various cultural characters. Hence, isolation methods are evidence-based research techniques that cannot be overlooked from the toolbox of techniques used for soil fungi identification (Tuininga et al. 2009, Gutleben et al. 2018, Libor et al. 2019).

Therefore, several factors must be considered when designing soil isolation experiments: the number of soil samples and horizons, collection and isolation protocol(s), isolation media(s), culture observations, and suitable preservation methods. Furthermore, the incorporation of expert knowledge and skills of taxonomists is also an essential factor for thorough investigations (Bills et al. 2004). A balance of the above factors will help ensure accuracy in results and minimize human errors (Mueller et al. 2004). In addition, understanding the limitations and challenges of the culture-dependent methods encourages researchers to develop traditional static methods in novel ways. Finally, advanced culture-dependent methods should be initiated more in unusual niches (Kazerooni et al. 2017, Wu et al. 2019).

Use of HTS in soil fungal studies

Metagenetics uses metabarcoding sequencing to examine the community composition of microorganisms. The nuclear ribosomal internal transcribed spacer (ITS) region is used as the primary barcode marker for fungi, followed by the small (SSU) and large subunit ribosomal ribonucleic acid (LSU) and their combinations (Taberlet et al. 2012, Ji et al. 2013, Chambergo & Valencia 2016, Gutleben et al. 2018, Nilsson et al. 2019, Tedersoo et al. 2020, Semenov 2021). Numerous sequences are derived from a single sample in the analysis and are present as OTUs (Schoch et al. 2012, Lindahl et al. 2013, Nilsson et al. 2019, Tedersoo et al. 2020). As a result, the HTS approach has been widely used in studies in the most diverse soil biomes (Tedersoo et al. 2014, Prosser 2015, Thompson et al. 2017, Tedersoo et al. 2020). Specifically, HTS methods have become affordable and capable of recovering soil fungi from large land areas, especially in habitats with extreme environmental conditions (Tedersoo et al. 2020), because organisms from oligotrophic soils in Antarctica, dry valleys, glaciers in pole regions, salty lands, and hypersaline microbial mats are difficult to be recovered *in vitro* (Semenov 2021). Therefore, HTS can reveal fungi, including rare to cryptic taxa, uncultivable fungi including *Archaeorhizomycetes*, *Cryptomycota*, *Zygomycotina*, and uncultivable symbiotic fungi such as mycorrhizal fungi and nematophagous fungi (Blackwell 2011, Rosling et al. 2011, Sota et al. 2014, Wu et al. 2019). HTS-generated data can also be used in ecological studies to map the diversity and distribution patterns of soil communities from different environmental conditions and land uses (Lumini et al. 2010, Salvioli & Bonfante 2013, Franzosa et al. 2015, Tedersoo et al. 2014, Wang et al. 2015, Asemaninejad et al. 2018, Kazerooni 2017, Schlatter et al. 2018, Bonito et al. 2019, Egidi et al. 2019).

Latest HTS findings – The Global Soil Mycobiome consortium dataset and endemicity of soil fungi

The GSMc dataset is a ready-to-use fungal HTS data derived from global soil sampling (Tedersoo et al. 2021). It provides an overview of the worldwide soil fungal diversity and distribution patterns, supplemented with macroecological metadata. Of all the recently published HTS data, GSMc dataset is the largest in the number of samples and geographical breadth. It recovered 1,157,667 OTUs and revealed the richness of the fungal kingdom. As per GSMc, belowground fungal functional guilds determined 124,616 OTUs of ectomycorrhizal (EcM), 75,530 OTUs of saprotrophs, 49,521 OTUs of unspecified saprotrophs, and 43.758 OTUs of unspecified pathotrophs (Tedersoo et al. 2021). In the most recent GSMc publication, Tedersoo et al. (2022) used the dataset to study the endemicity and vulnerability of soil fungal species. It revealed that belowground taxa are most vulnerable to droughts and high temperatures, resulting in the prevalence of the lifestyle shifting of the pathogenic, mutualistic, and free-living fungal groups. Biotrophic pathogens and EcM species can be highly vulnerable in case of loss of biotic niche due to environmental changes (Tedersoo et al. 2022). The authors suggested the need for habitat conservation, the inclusion of fungi in conservation frameworks to protect the endemic groups, and encouraged soil diversity assessment using HTS technology (Tedersoo et al. 2022).

Limitations of the HTS methods for soil fungal studies

Despite its wide application, HTS has several drawbacks. Chemical contaminations such as soil-inherent humic and fulvic acids can potentially inhibit enzymatic reactions affecting PCR-based DNA amplification or cloning procedures (Lombard et al. 2011, Lindahl et al. 2013). In the studies using HTS, the extraction aims to obtain a total DNA containing extracellular (exDNA) and intracellular DNA (iDNA). However, iDNA provides more reliable genetic information concerning the microbial communities than exDNA (Taberlet et al. 2012, Nagler et al. 2018, Nagler et al. 2021). exDNA can mask genetic information from the iDNA as the DNA portion from intact cells within the total DNA pool is undetermined. Therefore, HTS methods cannot distinguish living and dead materials or active and dormant organisms (Tuininga et al. 2009), thus reducing the reliability of data interpretation for the taxonomic composition of soil microbes (Nagler et al. 2021).

The lack of reference information and sequences in public databases, available bioinformatics tools, and higher error rates (5–13%) are the main limitation of HTS (Tedersoo et al. 2017, Mafune et al. 2019). Likewise, short-read sequences from environmental samples generated by HTS are challenging to align, assign and annotate for further study (Wu et al. 2019, Chethana et al. 2020), making it tough to assign fungal OTUs to lower taxonomic levels, resulting in numerous OTUs named under unclassified taxa (Hongsanan et al. 2018, Wu et al. 2019, Chethana et al. 2020). Comparing the Global Fungi dataset (Větrovský et al. 2020) by Baldrian et al. (2022) and GSMc by Tedersoo et al. (2021), the richness of *Zoopagomycota*, *Entomophthoromycota*, *Blastocladiomycota*, and *Kickxellomycota* showed grater levels of contradictions as a result of different sampling strategies and analytical biases such as the use of different primers for metabarcoding and lack of properly annotated reference sequences (Tedersoo et al. 2021).

In taxonomic assignments, as sequences from environmental samples cannot be correlated to a physical specimen, reproduced, or verified, there is thus a higher possibility for erroneous sequences or dark taxa (Tedersoo et al. 2017, Chethana et al. 2020). In addition, the storage, processing, integration, and interpretation of data using bioinformatics analysis for HTS are all considerably complex, requiring computer power and specialized personnel (Rincon-Florez et al. 2013, Sota et al. 2014, Nilsson et al. 2019, Wu et al. 2019).

Integrating culture and "genomics" techniques to understand the taxonomy and biodiversity of soil fungi

Combining culture-based results with molecular-based data can reduce inherent technical errors in HTS and data interpretations and reduce bias in culturing methods (Rincon-Florez et al. 2013, Dissanayake et al. 2018, Jayawardena et al. 2018, Wu et al. 2019, Wijayawardena et al. 2021). Genomics methods recover hidden unculturable diversity and thus can potentially help predesign isolation methodologies to obtain cultures of these taxa (Stefani et al. 2015, Chambergo & Valencia 2016, Gutleben et al. 2018). HTS can be used to identify fungal secondary metabolite biosynthesis gene clusters from environmental samples, but the cultivation of these strains is essential to obtain the products (Chambergo & Valencia 2016). Thus, a combination of methods can improve the characterization of taxa for biotechnological use. Bioactive compounds produced by soil fungi are used in bioremediation, food production, and pharmaceutical industries (Muller et al. 2013, Stefani et al. 2015, Gutleben et al. 2018, Hyde et al. 2019). In this regard, Stefani et al. (2015) used HTS to recover strains able to degrade hydrocarbons, of which most were unculturable and thus could not test their hydrocarbon degradation capacity (Stefani et al. 2015). Therefore, the combination of culture-dependent and -independent approaches may provide better insights (Anderson & Cairney 2014).

Conclusion and future perspectives

Fungi are abundant in soil micro-environments and have been found to be the most common of microorganisms (Tedersoo et al. 2021, 2022). They function significantly in mediating soil biological, chemical, and physical components and feature notably in global soil biodiversity levels, taxonomy, and biotechnology (Tedersoo et al. 2021, Bahram & Netherway 2022). However, exploration the diversity is a major challenge. High-throughput screening has estimated that there are up to 12 million species of fungi (Wu et al. 2019), and Tedersoo et al. (2021) recovered 722,682 fungal OTUs from soils globally. Detection and identification methods, therefore, pose numerous issues and challenges. Although detection by HTS has broadened the knowledge of soil fungi, most OTUs can only be assigned to the genus or higher levels because of short-read sequences, a lack of reference sequences, and error-prone results. Conversely, isolates from soil provide living mycelia, facilitating morphological observation, species-level identification, and sources for biotechnology and research. Soil plating, dilution, and baiting methods are widely used isolation methods. However, because different taxa may have specific requirements for their growth, these methods are not applicable for the isolation of total diversity.

The combination of culture-dependent and -independent methods will provide a dynamic understanding of soil dwellers (Hirsch et al. 2013, Stefani et al. 2015, Kazerooni et al. 2017, Hamm et al. 2020). Genomic approaches, including HTS, provide molecular data for soils across a large geographical area, which is beneficial for generating better insights and functioning as a guiding tool to initiate isolation studies (Wu et al. 2019). The development of novel isolation methods for uncultured soil fungi is needed to address the inherent problems and challenges of understanding the role of soil fungi.

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References

Abdullah SK, Saadullah AA. 2013 – Soil myco-biota at grapevine plantations in Duhok, North Iraq. Mesopotamia Journal of Agriculture 41, 402–412.

Abu-Mejdad NMJA. 2013 – Biochemical study for three keratins baiting and using it to isolation and identification some fungi from soil in Basrah, Iraq. International Journal of Engineering and Innovative Technology 3, 216–224.

Aghamirian MR, Ghiasian SA. 2013 – The prevalence of fungi in soil of Qazvin, Iran. Jundishapur Journal of Microbiology 6, 76–79. Doi 10.5812/jjm.4591

Aime MC, Miller AN, Aoki T, Bensch K et al. 2021 – How to publish a new fungal species, or name, version 3.0. IMA fungus 12, 1–15. Doi 10.1186/s43008-021-00063-1

Ali AM, Moghazy S, Shaban G, El-Sababty Z. 2009 – Heat-resistant fungi isolated from soil in minia governorate, Egypt. Assiut University Journal of Botany, Assiut University. 38, 93–106. Doi 10.25130/tjps.23.2018.147

Alidadi A, Vala SA, Jouzani GS. 2020 – *Botryotrichum iranicum* sp. nov. and *Trematosphaeria magenta* sp. nov. as two new species from Iran. Mycological Progress 19, 1575–1586. Doi 10.1007/s11557-020-01648-7

Altayyar IA, Osman NA, Elbreki MF, Ibrahim H et al. 2016 – Isolation and identification of soil keratinophilic fungi from different area in south of Libya. International Journal of Applied Medical and Biological Research 1, 27–32.

Alotaibi MO, Sonbol HS, Alwakeel SS. Suliman RS et al. 2020 – Microbial diversity of some sabkha and desert sites in Saudi Arabia. Saudi Journal of Biological Sciences 27, 2778–2789. Doi 10.1016/j.sjbs.2020.06.038

An ZQ, Hendrix JW, Hershman DE, Henson GT. 1990 – Evaluation of the "Most probable number" (Mpn) and wet-sieving methods for determining soil-borne populations of endogonaceous mycorrhizal fungi. Mycologia, 82, 576–581. Doi 10.1080/00275514.1990.12025932

Anandaraj M, Sarma YR. 1990 – A simple baiting technique to detect and isolate *Phytophthora capsici* ('*P. Palmivora*' MF4) from soil. Mycological Research 94, 1003–1004. Doi 10.1016/S0953-7562(09)81321-X

Anderson IC, Cairney JW. 2004 – Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environmental microbiology, 6, 769–779.

Asemaninejad A, Thorn RG, Branfireun B, Lindo Z. 2018 – Climate change favours specific fungal communities in boreal peatlands. Soil Biology and Biochemistry 120, 28–36. Doi 10.1016/j.soilbio.2018.01.029

Averill C, Werbin ZR, Atherton KF, Bhatnagar JM, Dietze MC. 2021 – Soil microbiome predictability increases with spatial and taxonomic scale. Nature Ecology & Evolution 5, 747–756. Doi 10.1038/s41559-021-01445-9

Azaz AD. 2003 – Isolation and identification of soil borne fungi in fields irrigated by GAP in Harran Plain using two isolation methods. Turkish Journal of Botany 27, 83–92.

Aziz N, Zainol N. 2018 – Isolation and identification of soil fungi isolates from forest soil for flooded soil recovery. IOP Conference Series: Materials Science and Engineering 342. Doi 012028. 10.1088/1757-899X/342/1/012028.

Bailey F, Gray NF. 1989 – The comparison of isolation techniques for nematophagous fungi from soil. Annals of Applied Biology 114, 125–132. Doi 10.1111/j.1744-7348.1989.tb06792.x

Baldrian P, Větrovský T, Lepinay C, Kohout P. 2022 – High-throughput sequencing view on the magnitude of global fungal diversity. Fungal Diversity, 114, 539–547. Doi 10.1007/s13225-021-00472-y

Ballhausen M, Veen J, Hundscheid M, de Boer W. 2015 – Methods for Baiting and Enriching Fungus-Feeding (Mycophagous) Rhizosphere Bacteria. Frontiers in Microbiology 6, 1416. Doi 10.3389/fmicb.2015.01416.

Basu S, Bose C, Ojha N, Das N et al. 2015 – Evolution of bacterial and fungal growth media. Bioinformation 11, 182. Doi 10.6026/97320630011182

Beuchat LR, Pitt JI. 1992 – Detection and enumeration of heat-resistant molds, in: Compendium for the Microbiological Examination of Foods (pp. 251–263). In Compendium of methods for the microbiological examination of foods, American Public Health Association, Washington D. C.

Bills GF, Christensen M, Powell M, Thorn G. 2004 – Saprobic soil fungi, p. 271–302. In Mueller GM, Bills GF, Foster MS. (ed.), Biodiversity of fungi, inventory and monitoring methods. Elsevier Academic Press, Oxford, England.

Bills GF, Arias RM, Reyes M, Heredia G. 2001 – *Merimbla humicoloides* sp. nov. from conifer forest soil of Veracruz state. Mexico. Mycological Research 105, 1273–1279. Doi 10.1016/S0953-7562(08)62000-6

Biswas R, Sarkar A. 2018 – 'Omics' Tools in Soil Microbiology: The State of the Art. In: Adhya T, Lal B, Mohapatra B, Paul D, Das S. (eds) Advances in Soil Microbiology: Recent Trends and Future Prospects. Microorganisms for Sustainability, vol 3. Springer, Singapore. Doi 10.1007/978-981-10-6178-3_3

Blackwell M. 2011 – The Fungi:1, 2, 3 … 5.1 million species? American Journal of Botany 98, 426–438. Doi 10.3732/ajb.1000298

Bonito GM, Benucci GMN, Hameed K, Weighill DP et al. 2019 – Fungal-bacterial networks in the *Populus rhizobiome* are impacted by soil properties and host genotype. Frontiers in Microbiology 10, 481. Doi 10.3389/fmicb.2019.00481

Bahram M, Netherway T. 2022 – Fungi as mediators linking organisms and ecosystems. FEMS Microbiology Reviews 46, 2, fuab058. Doi 10.1093/femsre/fuab058

Brierley WB, Jewson ST, Brierley M. 1928 – The quantitative study of soil fungi (pp. 48–71.). In Proceedings of the 1st International Congress of Soil Science, International Society of Soil Science, Washington, D.C.

Bridge P, Spooner B. 2001 – Soil fungi: diversity and detection. Plant and Soil 232, 147–154. Doi 10.1023/A:1010346305799

Bhunjun CS, Niskanen T, Suwannarach N. Wannathes N et al. 2022 – The numbers of fungi: are the most speciose genera truly diverse?. Fungal Diversity. Doi 10.1007/s13225-022-00501-4

Butler EJ. 1907 – An account of the genus *Pythium* and some *Chytridiaceae*. Memoirs of the Department of Agriculture in India 1, 1–162.

Calduch M, Gené J, Stchigel AM, Cano JF et al. 2004 – *Ramophialophora*, a new anamorphic genus of *Sordariales*. Studies in Mycology 50, 83–88.
Călin M, Răut I, Constantinescu-Aruxandei D, Doni MB et al. 2017 – Influence of culture conditions on growth of keratinophilic fungal strains. Scientific Bulletin. Series F. Biotechnologies 21, 173–178.
Cameron EK, Martins IS, Lavelle P, Mathieu J et al. 2018 – Global gaps in soil biodiversity data. Nature Ecology & Evolution, 2, 1042–1043. Doi 10.1038/s41559-018-0573-8
Carreiro MM, Koske RE. 1992 – Room temperature isolations can bias against selection of low temperature microfungi in temperate forest soils. Mycologia, 84, 886–900. Doi 10.1080/00275514.1992.12026221
Carvalhais LC, Dennis PG, Tyson GW, Schenk PM. 2012 – Application of meta transcriptomics to soil environments. Journal of Microbiological Methods 91, 246–251. Doi 10.1016/j.mimet.2012.08.011
Chambergo FS, Valencia EY. 2016 – Fungal biodiversity to biotechnology. Applied microbiology and biotechnology 100, 2567–2577. Doi 10.1007/s00253-016-7305-2
Chesters CGC. 1940 – A method for isolating soil fungi. Transaction of the British Mycological Society 2, 352–355.
Chesters CGC. 1948 – A contribution to the study of fungi in the soil. Transactions of the British Mycological Society. 30, 100–113. Doi 10.1016/S0007-1536(48)80038-0
Chesters CGC, Thornton RH. 1956 – A comparison of techniques for isolating soil fungi. Transactions of the British Mycological Society 39, 301–313. Doi 10.1016/S0007-1536(56)80015-6
Chethana KWT, Jayawardena RS, Hyde KD. 2020 – Hurdles in fungal taxonomy: Effectiveness of recent methods in discriminating taxa. Megataxa 1, 114–122. Doi 10.11646/megataxa.1.2.2
Collado J, Platas G, Paulus B, Bills GF. 2007 – High-throughput culturing of fungi from plant litter by a dilution-to-extinction technique. FEMS Microbiology Ecology 60, 521–533. Doi 10.1111/j.1574-6941.2007.00294.x
Das K, Lee SY, Jung HY. 2019 – *Cladophialophora lanosa* sp. nov., a new species isolated from soil. Mycobiology 47, 173–179. Doi 10.1080/12298093.2019.1611242
Das K, Lee SY, Choi HW, Eom AH et al. 2020 – Taxonomy of *Arthrinium minutisporum* sp. nov., *Pezicula neosporulosa,* and *Acrocalymma pterocarpi*: new records from soil in Korea. Mycobiology 48, 450–463. Doi 10.1080/12298093.2020.1830741
Dentinger BTM, Gaya E, O'Brien H, Suz LM et al. 2016 –Tales from the crypt: genome mining from fungarium specimens improves resolution of the mushroom tree of life, Biological Journal of the Linnean Society 117, 11–32. Doi 10.1111/bij.12553
Dissanayake AJ, Purahong W, Wubet T, Hyde KD et al. 2018 – Direct comparison of culture-dependent and culture-independent molecular approaches reveal the diversity of fungal endophytic communities in stems of grapevine (*Vitis vinifera*). Fungal Diversity 90, 85–107. Doi 10.1007/s13225-018-0399-3
Domsch KH, Gams W, Anderson TH. 1993 – Compendium of Soil Fungi. IHWVerlag Press.
Dorhout DL, Sappington TW, Lewis LC, Rice ME. 2011 – Flight behaviour of European corn borer infected with *Nosema pyrausta*. Journal of Applied Entomology 135, 25–37. Doi 10.1111/j.1439-0418.2010.01523.x
Durán P, Barra PJ, Jorquera MA, Viscardi S. 2019 – Occurrence of soil fungi in Antarctic pristine environments. Frontiers in Bioengineering and Biotechnology 7, 28. Doi 10.3389/fbioe.2019.00028
Egidi E, Delgado-Baquerizo M, Plett JM, Wang J et al. 2019 – A few Ascomycota taxa dominate soil fungal communities worldwide. Nature Communications 10, 2369. Doi 10.1038/s41467-019-10373-z
Elad Y, Chet I, Henis Y. 1981 – A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica 91, 59–67.

Foster MS, Mueller GM, Bills GF. 2011 – Biodiversity of Fungi: inventory and monitoring methods. Elsevier.

Fernandes ÉK, Keyser CA, Rangel DE, Foster RN et al. 2010 – CTC medium: A novel dodine-free selective medium for isolating entomopathogenic fungi, especially *Metarhizium acridum*, from soil. Biological Control 54, 197–205. Doi 10.1016/j.biocontrol.2010.05.009

Frac M, Hannula SE, Bełka M, Jędryczka M. 2018 – Fungal biodiversity and their role in soil health. Frontiers in Microbiology 9, 707. Doi 10.3389/fmicb.2018.00707

Fracetto GGM, Azevedo LCB, Fracetto FJC, Andreote FD et al. 2013 – Impact of Amazon land use on the community of soil fungi. Scientia Agricola 70, 59–67. Doi 10.1590/S0103-90162013000200001

Franzosa EA, Hsu T, Sirota-Madi A, Shafquat A et al. 2015 – Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. Nature Reviews Microbiology 13, 360–372. Doi 10.1038/nrmicro3451

Furuya K, Naito A. 1979 – An effective method for isolation of *Ascodesmis* from soil. Transactions of the Mycological Society of Japan 20, 171–175

Furuya K, Naito A. 1980 – Stimulation of ascospore germination by phenolic compounds in numbers of the *Sordariaceae*. Transactions of the Mycological Society of Japan (Japan). 21,77–85.

Garrett SD. 1981 – Soil fungi and soil fertility, 2nd edn, Oxford, England: Pergamon Press.

Gerdemann JW, Nicolson TH. 1963 – Spores of mycorrhizalendogone species extracted from soil by wet-sieving and decanting. Transactions of the British Mycological Society 46, 235–244.

Giudice MC, Reis-Menezes AA, Rittner GMG, Mota AJ et al. 2012 – Isolation of *Microsporum gypseum* in soil samples from different geographical regions of Brazil, evaluation of the extracellular proteolytic enzymes activities (keratinase and elastase) and molecular sequencing of selected strains. Brazilian Journal of Microbiology 43, 895–902. Doi 10.1590/S1517-83822012000300007

Ghodsalavi B, Svenningsen NB, Hao X, Olsson S et al. 2017 – A novel baiting microcosm approach used to identify the bacterial community associated with *Penicillium bilaii* hyphae in soil. PloS one 12, e0187116. Doi 10.1371/journal.pone.0187116

Gochenaur SE. 1964 – A modification of the immersion tube method for isolating soil fungi. Mycologia 56, 921–923. Doi 10.2307/3756658

Grossbard E. 1952 – Antibiotic production by fungi on organic manures and in soil. Microbiology 6, 295–310. Doi 10.1099/00221287-6-3-4-295

Gurusinghe S, Brooks TL, Barrow RA, Zhu X et al. 2019 – Technologies for the selection, culture and metabolic profiling of unique rhizosphere microorganisms for natural product discovery. Molecules 24, 1955. Doi 10.3390/molecules24101955

Guimarães LHS, Peixoto-Nogueira SC, Michelin M, Rizzatti ACS et al. 2006 – Screening of filamentous fungi for production of enzymes of biotechnological interest. Brazilian Journal of Microbiology 37, 474–480. Doi 10.1590/S1517-83822006000400014

Guevara-Suarez M, García D, Cano-Lira JF, Guarro J et al. 2020 – Species diversity in *Penicillium* and *Talaromyces* from herbivore dung, and the proposal of two new genera of penicillium-like fungi in *Aspergillaceae*. Fungal Systematics and Evolution 5, 39. Doi 10.3114/fuse.2020.05.03

Gutleben J, Chaib De Mares M, Van Elsas JD, Smidt H et al. 2018 – The multi-omics promise in context: from sequence to microbial isolate. Critical reviews in Microbiology, 44, 212–229. Doi 10.1080/1040841X.2017.1332003

Hamm PS, Mueller RC, Kuske CR, Porras-Alfaro A. 2020 – Keratinophilic fungi: Specialized fungal communities in a desert ecosystem identified using cultured-based and Illumina sequencing approaches. Microbiological Research 239, 126530. Doi 10.1016/j.micres.2020.126530

Hawksworth DL, Rossman AY. 1997 – Where are all the undescribed fungi? Phytopathology 87, 888–891. Doi 10.1094/PHYTO.1997.87.9.888

He MQ, Zhao RL, Liu DM, Denchev TT et al. 2022 – Species diversity of *Basidiomycota*. Fungal Diversity, 1– 45. Doi 10.1007/s13225-021-00497-3

Hibbett DS, Ohman A, Glotzer D, Nuhn M et al. 2011 – Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. Fungal Biology Reviews 25, 38–47. Doi 10.1016/j.fbr.2011.01.001

Hirsch J, Galidevara S, Strohmeier S, Devi KU et al. 2013 – Effects on diversity of soil fungal community and fate of an artificially applied *Beauveria bassiana* strain assessed through 454 pyrosequencing. Microbial Ecology 66, 608–620. Doi 10.1007/s00248-013-0249-5

Hodkinson ID, Wookey PA. 1999 – Functional ecology of soil organisms in tundra ecosystems: towards the future. Applied Soil Ecology 11, 111–126. Doi 10.1016/S0929-1393(98)00142-5

Holliday P, Mowat WP. 1963 – Foot rot of *Piper nigrum L*. (*Phytophthora palmivora*). Phytopathological Papers No. 5: Commonwealth Mycological Institute, Kew, Surrey, England.

Hongsanan A, Jeewon R, Purahong W, Xie N et al. 2018 – Can we use environmental DNA as holotypes? Fungal Diversity 92, 1–30. Doi 10.1007/s13225-018-0404-x

Hunter BB, Hoyes JV, Barnett HL. 1974 – The additions of aureomycin and chloramphenicol to various fungal agar media to prevent bacterial contamination. In Proceedings of the Pennsylvania Academy of Science (pp. 88–92). Pennsylvania Academy of Science.

Hyde KD, Dong Y, Phookamsak R, Jeewon R et al. 2020b – Fungal diversity notes 1151–1276: taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Diversity 100, 5–277. Doi 10.1007/s13225-020-00439-5

Hyde KD, Jeewon R, Chen YJ, Bhunjun CS et al. 2020c – The numbers of fungi: is the descriptive curve flattening? Fungal Diversity 103:219–271. Doi 10.1007/s13225-020-00458-2

Hyde KD, Norphanphoun C, Maharachchikumbura SSN, Bhat DJ et al. 2020a – Refined families of *Sordariomycetes*. Mycosphere 11, 305–1059. Doi 10.5943/mycosphere/11/1/7

Hyde KD, Xu JC, Rapior S, Jeewon R et al. 2019 – The amazing potential of fungi: 50 ways we can exploit fungi industrially. Fungal Diversity 97, 1–136. Doi 10.1007/s13225-019-00430-9

Jacoby R, Peukert M, Succurro A, Koprivova A et al. 2017 – The role of soil microorganisms in plant mineral nutrition–current knowledge and future directions. Frontiers in Plant Science 8, 1617. Doi 10.3389/fpls.2017.01617

Jayawardena RS, Purahong W, Zhang W, Wubet T et al. 2018 – Biodiversity of fungi on *Vitis vinifera* L. revealed by traditional and high-resolution culture-independent approaches. Fungal Diversity 90, 1–84. Doi 10.1007/s13225-018-0398-4

Jeewon R, Hyde KD. 2007 – Detection and Diversity of Fungi from Environmental Samples: Traditional Versus Molecular Approaches. In: Varma A, Oelmüller R. (eds) Advanced Techniques in Soil Microbiology. Soil Biology, vol 11. Springer, Berlin, Heidelberg. Doi 10.1007/978-3-540-70865-0_1

Ji Y, Ashton L, Pedley SM, Edwards DP et al. 2013 – Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. Ecology Letters 16, 1245–1257. Doi 10.1111/ele.12162

Junier P, Cailleau G, Palmieri I, Vallotton C et al 2021 – Democratization of fungal highway columns as a tool to investigate bacteria associated with soil fungi, FEMS Microbiology Ecology 97, fiab003. Doi 10.1093/femsec/fiab003

Jurburg SD, Keil P, Singh BK, Chase JM. 2021 – All together now: Limitations and recommendations for the simultaneous analysis of all eukaryotic soil sequences. Molecular Ecology Resources 21, 1759–1771. Doi 10.1111/1755-0998.13401

Kaewchai S, Soytong K, Hyde KD. 2009 – Mycofungicides and fungal biofertilizers. Fungal Diversity 38, 25–50.

Katan T. 1985 – Heat activation of dormant ascospores of *Talaromyces flavus*. Transactions of the British Mycological Society, 84, 748–750. Doi 10.1016/S0007-1536(85)80137-6

Kazerooni EA, Maharachchikumbura SS, Rethinasamy V, Al-Mahrouqi H et al. 2017 – Fungal diversity in tomato rhizosphere soil under conventional and desert farming systems. Frontiers in Microbiology 8, 1462. Doi 10.3389/fmicb.2017.01462

Kohlmeyer J, Kohlmeyer E. 1972 – Permanent microscopic mounts. Mycologia 64(3), 666–669.

Kuehn HH, Orr GF. 1962 – Tolerance of certain fungi to actidione and its use in isolation of *Gymnoascaceae*. Sabouraudia 1, 220–229. Doi 10.1080/00362176285190421

Kueh TK, Khew KL. 1982 – Survival of *Phytophthora palmivora* in soil and after passing through alimentary canals of snails. Plant Disease 66, 897–899.

Kumar PR, Hemanth G, Niharika PS, Kolli SK. 2015 – Isolation and identification of soil mycoflora in agricultural fields at Tekkali Mandal in Srikakulam District. International Journal of Advances in Pharmacy, Biology and Chemistry 4(2), 484–490.

Knudsen GR, Dandurand LMC. 2014 – Ecological complexity and the success of fungal biological control agents. Advances in Agriculture 2014. Doi 10.1155/2014/542703

Kutateladze LY, Zakariashvili NG, Jobava MD, Burduli TA et al. 2016 – Microscopic fungi spread in different types of soils in Western Georgia. Annals of Agrarian Science 14, 227–232. Doi 10.1016/j.aasci.2016.08.007

Libor B, Harms H, Kehraus S, Egereva E et al. 2019 – Isolation of fungi using the diffusion chamber device FIND technology. Beilstein Journal of Organic Chemistry 15, 2191–2203. Doi 10.3762/bjoc.15.216

Liimatainen K, Niskanen T, Dima B, Ammirati JF et al. 2020 – Mission impossible completed: unlocking the nomenclature of the largest and most complicated subgenus of *Cortinarius*, *Telamonia*. Fungal Diversity, 104, 291–331. Doi 10.1007/s13225-020-00459-1

Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K et al. 2013 – Fungal community analysis by high-throughput sequencing of amplified markers - a user's guide. The New Phytologist 199, 288–299. Doi 10.1111/nph.12243

Lombard N, Prestat E, Elsas JD, Simonet P. 2011 – Soil-specific limitations for access and analysis of soil microbial communities by metagenomics. FEMS Microbiology Ecology 78, 31–49. Doi 10.1111/j.1574-6941.2011.01140.x

Lumini E, Orgiazzi A, Borriello R, Bonfante P et al. 2010 – Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. Environmental Microbiology 12, 2165–2179. Doi 10.1111/j.1462-2920.2009.02099.x

Mafune KK, Godfrey BJ, Vogt DJ, Vogt KA. 2019 – A rapid approach to profiling diverse fungal communities using the MinION nanopore sequencer. BioTechniques 68, 72–78. Doi 10.2144/btn-2019-0072

Maharachchikumbura SSN, Chen Y, Ariyawansa HA, Hyde KD et al. 2021 – Integrative approaches for species delimitation in *Ascomycota*. Fungal Diversity 109, 155–179. Doi 10.1007/s13225-021-00486-6

Mankau R. 1975 – A semi-quantitative method for enumerating and observing parasites and predators of soil nematodes. Journal of Nematology 7, 119–122.

Manoch L. 1998 – Biodiversity of soil fungi in Thailand, pp. 126–140. In Proceedings of the Asia-Pacific Mycological Conference on Biodiversity and Biotechnology, Hua Hin

Marin-Felix Y, Hernández-Restrepo M, Iturrieta-González I, García D et al. 2019 – Genera of phytopathogenic fungi: GOPHY 3, Studies in Mycology, 94, 1–124. Doi 10.1016/j.simyco.2019.05.001.

Masoudi A, Wang M, Zhang X, Wang C et al. 2020 – Meta-analysis and evaluation by insect-mediated baiting reveal different patterns of Hypocrealean entomopathogenic fungi in the soils from two regions of China. Frontiers in Microbiology 11, p. 1133. Doi 10.3389/fmicb.2020.01133

Meyling NV. 2007 – Methods for isolation of entomopathogenic fungi from the soil environment. Laboratory manual, Department of Ecology, Faculty of Life Sciences, University of Copenhagen, Copenhagen, 18 pp.

Muller EEL, Glaab E, May P, Vlassis N. 2013 – Condensing the omics fog of microbial communities. Trends in microbiology 21, 325–333. Doi 10.1016/j.tim.2013.04.009
Mueller GM, Bills GF, Foster MS. 2004 – Biodiversity of fungi: inventory and monitoring methods. Elsevier Academic Press, San Diego.
Mueller KE, Durrell L. 1957. – Sampling tubes for soil fungi. Phytopathology 47, 243.
Mukerji KG. 2002 – Soil Microbes. Techniques in Mycorrhizal Studies 7–13. Doi 10.1007/978-94-017-3209-3_2
Nagler M, Insam H, Pietramellara G, Ascher-Jenull J. 2018 – Extracellular DNA in natural environments: Features, relevance and applications. Applied Microbiology and Biotechnology, 102, 6343–6356. Doi 10.1007/s00253-018-9120-4
Nagler M, Podmirseg SM, Mayr M, Ascher-Jenull J et al. 2021 – The masking effect of extracellular DNA and robustness of intracellular DNA in anaerobic digester NGS studies: A discriminatory study of the total DNA pool. Molecular Ecology 30, 438–450. Doi 10.1111/mec.15740
Nilsson RH, Anslan S, Bahram M, Wurzbacher C et al. 2019 – Mycobiome diversity: high-throughput sequencing and identification of fungi. Nature Reviews Microbiology 17, 95–109. Doi 10.1038/s41579-018-0116-y
O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM et al. 2005 – Applied and Environmental Microbiology 71, 5544–5550. Doi 10.1128/AEM.71.9.5544-5550.2005
Orgiazzi A, Bardgett RD, Barrios E. 2016 – Global soil biodiversity atlas. European Commission.
Pacioni G. 1991 – Wet-sieving and decanting technique for spore extraction. Methods in Microbiology 23, 317–322.
Park MS, Lee JW, Kim SH, Park JH et al. 2020 – *Penicillium* from Rhizosphere Soil in Terrestrial and Coastal Environments in South Korea. Mycobiology 48, 431–442. Doi 10.1080/12298093.2020.1823611
Pakshir K, Ghiasi MR, Zomorodian K, Gharavi AR. 2013 – Isolation and molecular identification of keratinophilic fungi from public parks soil in Shiraz, Iran. BioMed Research International 2013. Doi 10.1155/2013/619576
Parkinson D, Kendrick WB. 1960 – Investigations of soil microhabitats. In: Ecology of Soil Fungi. Pp. 22–28. Liverpool University Press.
Parkinson D, Williams ST. 1960 – A method for isolating fungi from soil microhabitats. Plant and Soil 13, 347–355. Doi 10.1007/BF01394646
Parkinson D, Thomas A. 1965 – A comparison of methods for the isolation of fungi from rhizospheres. Canadian journal of Microbiology 11, 1001–1007. Doi 10.1139/m65-133
Peršoh D, Stolle N, Brachmann A, Begerow D et al. 2018 – Fungal guilds are evenly distributed along a vertical spruce forest soil profile while individual fungi show pronounced niche partitioning. Mycological Progress 17, 925–939. Doi 10.1007/s11557-018-1405-6
Prosser JI. 2015 – Dispersing misconceptions and identifying opportunities for the use of 'omics' in soil microbial ecology. Nature Reviews Microbiology 13, 439–446. Doi 10.1038/nrmicro3468
Qiu M, Zhang R, Xue C, Zhang S et al. 2012 – Application of bio-organic fertilizer can control Fusarium wilt of cucumber plants by regulating microbial community of rhizosphere soil. Biology and Fertility of Soils 48, 807–816. Doi 10.1007/s00374-012-0675-4
Raja HA, Miller AN, Pearce CJ, Oberlies NH. 2017 – Fungal identification using molecular tools: a primer for the natural products research community. Journal of Natural Products, 80, 756–770. Doi 10.1021/acs.jnatprod.6b01085
Rao S, Hyde KD, Pointing SB. 2013 – Comparison of DNA and RNA, and cultivation approaches for the recovery of terrestrial and aquatic fungi from environmental samples. Current Microbiology 66, 185–191. Doi 10.1007/s00284-012-0256-7
Ramachandran N, Sarma YR, Nambiar KN. 1986 – Spatial distribution of *Phytophthora palmivora*-MF4 in the root zones of *Piper nigrum*. Indian Phytopathology 39, 414–417.

Ramadan NA, Ismael HM. 2011 – Isolation of fungi seasonally from soil, air and water in villages in "Binary Safeen". In The 4th International Scientific Conference of Salahaddin University-Erbil 1, 666–612.

Ramakrishnan K. 1955 – Some aspects of soil fungal ecology. Proceedings: Plant Sciences, 41, 110–116. Doi 10.1007/BF03050034

Ramesh R, Thamilvanan D, Ramkumar A, Balakumar BS et al. 2019 – Bio-Activity of Secondary Metabolites extracted from soil fungi. International Journal of Scientific Research and Reviews 8, 887–901.

Rincon-Florez VA, Carvalhais LC, Schenk PM. 2013 – Culture-independent molecular tools for soil and rhizosphere microbiology. Diversity 5, 581–612. Doi 10.3390/d5030581

Rosling A, Cox F, Cruz-Martinez K, Ihrmark K et al. 2011 – *Archaeorhizomycetes*: unearthing an ancient class of ubiquitous soil fungi. Science 333(6044), 876–879. Doi 10.1126/science.1206958

Rosas-Medina M, Maciá-Vicente JG, Piepenbring M. 2019 – Diversity of fungi in soils with different degrees of degradation in Germany and Panama. Mycobiology 1–9. Doi 10.1080/12298093.2019.1700658

Salvioli A, Bonfante P. 2013 – Systems biology and "omics" tools: a cooperation for next-generation mycorrhizal studies. Plant Science, 203, 107–114. Doi 10.1016/j.plantsci.2013.01.001

Samson RA, Luiten B. 1975 – *Eleutherascus tuberculatus*, a new heat resistant ascomycete. Trans Br Mycol Soc 64, 338–340

Satyanarayana T, Deshmukh SK, Deshpande MV. 2019 – (Eds.). Advancing frontiers in mycology and mycotechnology: basic and applied aspects of fungi. Springer Nature. Doi 10.1007/978-981-13-9349-5_3

Santos JAD, Meyer E, Sette LD. 2020 – Fungal community in Antarctic soil along the retreating Collins Glacier (Fildes Peninsula, King George Island). Microorganisms 8, 1145. Doi 10.3390/microorganisms8081145

Sastry MML, Hedge RK. 1988 – Survival of *Phytophthora palmivora*. India Phytopathology 41, 118–121.

Schlatter DC, Kahl K, Carlson B, Huggins DR et al. 2018 – Fungal community composition and diversity vary with soil depth and landscape position in a no-till wheat-based cropping system. FEMS Microbiology Ecology 94, fiy098. Doi 10.1093/femsec/fiy098

Schmidt PA, Bálint M, Greshake B, Bandow C et al. 2013 – Illumina metabarcoding of a soil fungal community. Soil Biology and Biochemistry 65, 128–132. Doi 10.1016/j.soilbio.2013.05.014

Schoch CL, Seifert KA, Huhndorf S, Robert V et al. 2012 – Fungal Barcoding Consortium. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences 109, 6241–6246. Doi 10.1073/pnas.1117018109

Selbmann L, Stoppiello GA, Onofri S, Stajich JE et al. 2021 – Culture-dependent and amplicon sequencing approaches reveal diversity and distribution of black fungi in Antarctic cryptoendolithic communities. Journal of Fungi 7, 213.

Semenov MV. 2021 – Metabarcoding and metagenomics in soil ecology research: Achievements, challenges, and opportunities. Biology Bulletin Reviews 11, 40–53. Doi 10.1134/S2079086421010084

Sena HH, Sanches MA, Rocha DFS, Segundo F et al. 2018 – Production of bio-surfactants by soil fungi isolated from the Amazon forest. International Journal of Microbiology, 2018. Doi 10.1155/2018/5684261

Senanayake IC, Pem D, Rathnayaka AR, Wijesinghe SN et al. 2022 – Predicting global numbers of teleomorphic ascomycetes. Fungal Diversity 1–42. Doi 10.1007/s13225-022-00504-1

Senanayake IC, Rathnayaka AR, Marasinghe DS, Calabon MS et al. 2020 – Morphological approaches in studying fungi: collection, examination, isolation, sporulation and preservation. Mycosphere 11, 2678–2754. Doi 10.5943/mycosphere/11/1/20

Silva J, Freixo R, Gibbs P, Teixeira P. 2011 – Spray-drying for the production of dried cultures. International Journal of Dairy Technology 64, 321–335.

Simon A, Bindschedler S, Job D, Wick LY et al. 2015 – Exploiting the fungal highway: development of a novel tool for the in-situ isolation of bacteria migrating along fungal mycelium. FEMS Microbiology Ecology 91, fiv116. Doi 10.1093/femsec/fiv116

Simpanya MF, Baxter M. 1996 – Isolation of fungi from soil using the keratin-baiting technique. Mycopathologia 136, 85–89. Doi 10.1007/BF00437500

Sharma L, Oliveira I, Torres L, Marques G. 2018 – Entomopathogenic fungi in Portuguese vineyards soils: Suggesting a '*Galleria*-Tenebrio-bait method'as bait-insects *Galleria* and *Tenebrio* significantly underestimate the respective recoveries of *Metarhizium* (*robertsii*) and *Beauveria* (*bassiana*). MycoKeys 38, 1. Doi 10.3897/mycokeys.38.26790

Shukia P, Shukla CB, Kango N, Shukla A. 2003 – Isolation and characterization of a dermatophyte, *Microsporium gypseum* from poultry farm soils of Rewa (Madhya Pradesh), India. Pakistan Journal of Biological Sciences, 6, 622–625.

Sota T, Kagata H, Ando Y, Utsumi S et al. 2014 – Metagenomic approach yields insights into fungal diversity and functioning. In Species diversity and community structure (pp. 1–23). Springer, Tokyo. Doi 10.1007/978-4-431-54261-2_1

Srinath J, Bagyaraj DJ, Satyanarayana BN. 2003 – Enhanced growth and nutrition of micropropagated *Ficus benjamina* to *Glomus mosseae* co-inoculated with *Trichoderma harzianum* and *Bacillus coagulans*. World Journal of Microbiology and Biotechnology, 19, 69–72. Doi 10.1023/a:1022569702845

Stefani FO, Bell TH, Marchand C, de la Providencia et al. 2015 – Culture-dependent and-independent methods capture different microbial community fractions in hydrocarbon-contaminated soils. PloS one 10, e0128272. Doi 10.1371/journal.pone.0128272

Sudhakar N, Shanmugam H, Kumaran S, Coelho A et al. 2018 – Omics approaches in fungal biotechnology: Industrial and medical point of view. In Omics Technologies and Bio-engineering (pp. 53–70). Academic Press. Doi 10.1016/B978-0-12-815870-8.00004-8

Sundaram BM. 1977 – Fungal flora of rice field soils. In Proceedings of the Indian Academy of Sciences-Section B (85, 90–99). Springer India. Doi 10.1007/BF03050906

Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH. 2012 – Environmental DNA. Molecular Ecology 21, 1789–1793. Doi 10.1111/j.1365-294X.2012.05542.x

Tedersoo L, Anslan S, Bahram M, Kõljalg U, Abarenkov K. 2020 – Identifying the 'unidentified'fungi: a global-scale long-read third-generation sequencing approach. Fungal Diversity 103, 273–293. Doi 10.1007/s13225-020-00456-4

Tedersoo L, Bahram M, Puusepp R, Nilsson RH, James TY. 2017 – Novel soil-inhabiting clades fill gaps in the fungal tree of life. Microbiome 5, 42. Doi 10.1186/s40168-017-0259-5

Tedersoo L, Bahram M, Põlme S, Kõljalg U et al. 2014 – Global diversity and geography of soil fungi. Science 346, (6213), Doi 1078 1256688/1-1256688/10. Doi 10.1126/science.1256688

Tedersoo L, Mikryukov V, Anslan S, Bahram M et al. 2021 – The Global Soil Mycobiome consortium dataset for boosting fungal diversity research. Fungal Diversity 111, 573–588. Doi 10.1007/s13225-021-00493-7

Tedersoo L, Mikryukov W, Zizka A, Bahram M et al. 2022 – Towards understanding diversity, endemicity and global change vulnerability of soil fungi. bioRxiv (Preprint). Doi 10.1101/2022.03.17.484796

Thompson LR, Sanders JG, McDonald D, Amir A et al. 2017 – A communal catalogue reveals earth's multiscale microbial diversity Nature, 551, pp. 457–463. Doi 10.1038/nature24621

Thornton RH. 1952 – The screened immersion plate. A method of isolating soil microorganisms. Research 5, 190–191

Triffitt MJ. 1935 – On cyst-like bodies, resembling cysts of *Heterodera schachtii* of common occurrence in British soils. Helminth. 13, 59–66.
Tsao PH. 1970 – Selective media for isolation of pathogenic fungi. Annual Review of Phytopathology 8, 157–186. Doi 10.1146/annurev.py.08.090170.001105
Tsao PH, Ocana G. 1969 – Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. Nature 223, 636–638. Doi 10.1038/223636a0
Tsudome M, Deguchi S, Tsujii K, Ito S et al. 2009 – Versatile solidified nano fibrous cellulose-containing media for growth of extremophiles. Applied and Environmental Microbiology 75, 4616–4619. Doi 10.1128/AEM.00519-09
Tuininga AR, Miller JL, Morath SU, Daniels TJ et al. 2009 – Isolation of entomopathogenic fungi from soils and ixodes scapularis (acari: ixodidae) ticks: prevalence and methods. Journal of Medical Entomology, 46, 557–565. Doi 10.1603/033.046.0321
Van Elsas JD, Trevors JT, Rosado AS, Nannipieri P. 2019 – (Eds.). Modern soil microbiology. CRC press. (p. 421–430).
Veen KH, Ferron P. 1966 – A selective medium for the isolation of *Beauveria tenella* and of *Metarhizium anisopliae*. Journal of Invertebrate Pathology 8, 268–269.
Větrovský T, Morais D, Kohout P, Lepinay C et al. 2020 – GlobalFungi, a global database of fungal occurrences from high-throughput-sequencing metabarcoding studies. Sci Data, 7, 1–14. Doi 10.1038/s41597-020-0567-7
Vu D, Groenewald M, de Vries M, Gehrmann T et al. 2019 – Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation, Studies in Mycology 92, 135–154. Doi 10.1016/j.simyco.2018.05.001
Wagg C, Schlaeppi K, Banerjee S, Kuramae EE et al. 2019 – Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nature Communications 10, 1–10. Doi 10.1038/s41467-019-12798-y
Waksman SA. 1922 – A method for counting the number of fungi in the soil. Journal of Bacteriology 7(3), 339.
Wang JT, Zheng YM, Hu HW, Zhang LM et al. 2015 – Soil pH determines the alpha diversity but not beta diversity of soil fungal community along altitude in a typical Tibetan forest ecosystem. Journal of Soils and Sediments 15, 1224–1232. Doi 10.1007/s11368-015-1070-1
Warcup JH. 1950 – The soil-plate method for isolation of fungi from soil. Nature 166, 117. Doi 10.1038/166117b0
Warcup JH. 1951 – Soil-steaming: a selective method for the isolation of ascomycetes from soil. Transactions of the British Mycological Society 34, 515–518. Doi 10.1016/S0007-1536(51)80035-4.
Warcup JH, Baker KF. 1963 – Occurrence of dormant ascospores in soil. Nature 197, 1317. Doi 10.1038/1971317a0
Warcup JH. 1955 – Isolation of fungi from hyphae present in soil. Nature 175, 953–954. Doi 10.1038/175953b0
Wijayawardene NN, Bahram M, Sánchez-Castro I, Dai DQ et al. 2021 – Current insight into culture-dependent and culture-independent methods in discovering ascomycetous taxa. Journal of Fungi 7, 703. Doi 10.3390/jof7090703
Wijayawardene NN, Phillips AJ, Pereira DS, Da DQ et al. 2022 – Forecasting the number of species of asexually reproducing fungi (*Ascomycota* and *Basidiomycota*). Fungal Diversity 1–28. Doi 10.1007/s13225-022-00500-5
Wu B, Hussain M, Zhang W, Stadler M et al. 2019 – Current insights into fungal species diversity and perspective on naming the environmental DNA sequences of fungi. Mycology 10, 127–140. Doi 10.1080/21501203.2019.1614106
Yang C, Liu N, Zhang Y. 2019 – Soil aggregates regulate the impact of soil bacterial and fungal communities on soil respiration. Geoderma 337, 444–452. Doi 10.1016/j.geoderma.2018.10.002

Yasanthika WAE, Wanasinghe DN, Karunarathna SC, Bhat DJ et al. 2020 – Two new *Sordariomycetes* records from forest soils in Thailand. Asian Journal of Mycology 3, 456–472. Doi 10.5943/ajom/3/1/16

Zhang Y, Mo M, Yang L, Mi F et al. 2021 – Exploring the species diversity of edible mushrooms in Yunnan, Southwestern China, by DNA barcoding. Journal of Fungi 7, 310. Doi 10.3390/jof7040310