

Where to Find? A Report for Some Terrestrial Fungal Isolates, and Selected Applications Using Fungal Secondary Metabolites



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Abstract

Biodiversity of mycobiota is getting more attention, since fungi and their metabolites represent the future alternatives of many currently used chemical compounds. In this review, the fungal mycobiota associated with leaves of some plants were discussed comparing the literatures focusing on some economically important plants such as wheat, sugarcane, and some medical plants. Furthermore, the role of some fungi as promising small factories that could be used for industrial applications such as myco-diesel oil production, and agricultural applications such as plant growth promoters

Keywords: Fungal Biodiversity; Isolation; Identification; Phylloplane fungi; Mycobiota; Medicinal Plant; Myco-Diesel

Introduction

Biodiversity describes the variability among living organisms within a given ecosystem, and it is often used to measure the health of biological systems. Documenting and describing fungal diversity is an extremely difficult mission that requires years of experience due to their ability to appear in various morphologies from a single individual, and their confusing similarity in appearance to non closely-related taxa [1]. There are many methods used from decades to analyze fungal biodiversity. The classical methods rely on direct observation of fungi. These methods are cheaper and need no specific equipment in comparison with molecular based methods which rely on sampling fungal DNA. Classical methods are commonly used specially in developing countries, which usually cannot afford applying molecular methods. Classical methods are hence accompanied by doing many investigations, and record fungi over a specific area and /or amount of substrate [2,3]. Putting also in consideration ecological variables, such as pH, soil nutrients composition, age of the plant, time of the year, and some other weather-related variables [4].

Fungi are ubiquitous, and new species can be isolated even from the most well studied areas [5]. The word phylloplane point to the surface of plant leaves which represents a complex terrestrial habitat and so studying phylloplane mycobiota is concerned with the fungal flora growing on the surface of plant leaves [3]. Many studies focused on investigating phylloplane fungal flora of several plants growing or cultivated in Egypt and other parts of the world

[6-13]. There are two groups of phylloplane fungi: residents and casuals. Residents multiply on the surface of healthy leaves without harming the host plant or affecting it. While, casuals though existing on the surface of the leaf, they cannot grow on it. [14,15]. Studying biodiversity of mycobiota is very important for identifying and documenting changes and similarities between species. Moreover, various novel natural compounds can be isolated and identified from such mycobiota with promising potential biological, medical and industrial applications [16]. In this review, phylloplane mycobiota from some economically important plants and some medicinal plants were described. Moreover, using some fungal strains as small factories to produce valuable compounds involved in many applications was discussed.

Isolation of Phylloplane Mycobiota

A common method for isolation of phylloplane mycobiota, the agar plate method, was described by Elkhateeb [4], include washing leaves of the plant several times with distilled water, and dry them between sterile filter paper, followed by cutting the leaf into equal segments (about 1cm each). Five segments were placed in each plate on the surface of the agar medium supplemented with appropriate antibacterial. Then, the plates were incubated at 28°C ± 1 for 7-10 days, and the developing fungi were counted, identified, and calculated as colony forming unit "cfu" per 100 segments of fresh leaves.

Another commonly used method is moist chamber plate's method [17], which doesn't require using any type of medium for the growth of Phylloplane fungi. In this method the leaf segments of 1 Cm square, both sterile and unsterile were placed separately on the moist chamber plates using a sterile forceps and pressed gently, followed by incubation at 25±3°C in the incubation chamber for 7 to 21 days. But starting from the fourth day monitoring plates is very important in order to check the growth of fungi. The fungi on moist chamber were then counted.

Mycobiota from Economically Important Plants

Wheat is one of the most economically important plants worldwide. Many fungal genera were listed to be isolated from wheat. Abdel-Hafez [18] reported that the most common phylloplane genera isolated from wheat leaves cultivated in Saudi Arabia were *Cladosporium*, *Aspergillus*, and *Alternaria*, which came in accordance with Abdel-Hafez et al. [19] who reported the same genera *Alternaria*, *Aspergillus*, and *Cladosporium*, but he also added *Cochliobolus*, *Gibberella*, *Nectria*, and *Penicillium*. Solated to the list of common mycobiota isolated from wheat fields in El-Kharga Oasis, Western desert, Egypt. On the other hand, Mazen et al. [20] nominated the most common species isolated from phylloplane of wheat plants as *Aspergillus niger*, *Penicillium corylophilum* and *Alternaria alternata*.

Mycobiota biodiversity isolated from wheat were found to differ according to the plant part they were isolated from. Larran et al. [21] reported *Rhodotorula rubra*, *Alternaria alternata*, *Cladosporium herbarum* and *Epicoccum nigrum* as the predominant species isolated from wheat (*Triticum aestivum* L.). Whereas Larran et al. [22] recorded *Alternaria*, *Penicillium*, *Stemphylium*, *Fusarium*, *Aspergillus*, *Ulocladium*, *Epicoccum*, *Acremonium* and *Pythium* sp. to be the species highly colonizing in crown tissues, and *Alternaria*, *Stemphylium*, *Penicillium*, *Epicoccum*, *Ulocladium*, *Nigrospora*, *Torula*, *Septonema*, *Aspergillus* and *Phaeoseptoria* spp. as species predominantly colonizing leaves, while *Alternaria*, *Stemphylium*, *Penicillium*, *Cladosporium*, *Ulocladium*, *Epicoccum*, *Fusarium*, *Popularia*, *Nigrospora* and *Trichoderma* spp. as genera commonly isolated from ears of wheat.

Surveying mycobiota associated with sugarcane revealed that *Aspergillus flavus*, *A. niger*, *Cochliobolus spicifer*, *Fusarium oxysporum*, *Gibberella fujikuroi*, *Nectria haematococca* and *Penicillium chrysogenum* were the most common species [23,24] studied the fungal biota of leaf surface of banana and recorded the most common genera as *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Cochliobolus*, *Curvularia*, *Gibberella*, *Memnoniella*, *Mycosphaerella*, *Setosphaeria* and *Stachybotrys*. Surveying mycobiota on grapevine plantations in Sahel-Saleem City, Assuit Governorate, Egypt, predominated *Aspergillus niger* and *A. aculeatus*, *Penicillium* count came second after *Fusarium*. *Penicillium*. *Purpurogenum* and *Fusarium oxysporum* were the common species [25].

Phylloplane Mycobiota from Medicinal Plant

Prabakaran et al. [15] Survey on the occurrence of phylloplane fungi on leaves surface of three important medicinal plants such as *Ocimum sanctum* (holy basil), *Phyllanthus amarus* (sleeping

plant), and *Azadirachta indica* (neem tree), revealed predominance of *Aspergillus flavus*, *Penicillium expansum*, *Fusarium semitectum*, *Fusarium oxysporum* in the phylloplane of *Ocimum sanctum*. On the other hand, *Scopulariopsis* sp. was isolated from the phylloplane of *Phyllanthus amarus*, whereas *Penicillium janthinellum*, *Aspergillus fumigulosus*, *Aspergillus* sp., *Curvularia lunata* and *Fusarium moniliforme* were isolated from the phylloplane of *Azadirachta indica*. Nayak [17] studied mycobiota isolated from *Tinospora cordifolia* (heart-leaved moonseed), and nominated the species *Alternaria alternata*, *Aspergillus niger*, *Cladosporium herbarum*, *Penicillium chrysogenum* as dominant fungal taxa among the recorded species.

Nayak [26] described mycobiota isolated from leaves of *Solanum nigrum* using two different techniques, agar plate method and moist chamber plates method. He reported isolation of 16 fungi under 10 genera from both used methods. In agar plate technique, *Penicillium digitatum*, *Aspergillus awamori*, *Alternaria* were isolated but, by using moist chamber technique, *Torula herbarum*, *Trichoderma harzianum* and *Aspergillus flavus* were isolated from the leaf samples. *Alternaria* sp., *Cladosporium herbarum*, *Aspergillus awamori*, *A. flavipes*, *A. Niger*, *Monascus* sp, *Penicillium citrinum*, *P. digitatum*, *Torula herbarum* and *Trichoderma harzianum* were identified as phylloplane fungi.

Fungal Secondary Metabolites with Beneficial Properties

It is already known that fungi secrete a wide range of secondary metabolites which represent an excellent source of compounds with promising applications for industry, medicine, and agricultural fields. For example, *Epicoccum* Sp. is known to secrete many secondary metabolites such as epicorazines which has an antibacterial activity [27,28]. *Trichoderma* Sp. is widely used as biofertilizers and biocontrol agents in agriculture due to its secreted secondary metabolites that exert different biological activities towards plants and other microbes [29]. *T. harzianum* produces an important metabolite, harzianic acid (HA) which promotes plant growth [30]. Another metabolite was isolated from the culture filtrate of a *T. harzianum* strain a, termed isoharzianic acid (iso-HA), which has antifungal activities In vitro against *Sclerotinia sclerotiorum* and *Rhizoctonia solani*. [30-39].

Oil Production

The current energy crisis has highlighted the importance of searching for potent alternative oils and for synthesis of microbial lipids, which represent the raw material for biodiesel production [40]. Many fungi can accumulate high level of lipids and grow as pellets that can easily be harvest from cell broth [41]. One of the most potent fungus in oil production is *Mucor circinelloides*. Pellet formation in submerged fungal cultivation of *M. circinelloides* depends strongly on adjusting pH during cell growth [42]. Many agricultural wastes such as Sugar beet pulp (SBP) can be used as substrate for production of fungal oil from *Mucor circinelloides* and *Aspergillus oryzae*, which is an economical way to offer an alternative to oil to be used as an energy source. Another fungus, *Cunninghamella japonica*, was reported as a promising lipid producer [43-45].

Conclusion

As described in this review, even the well-studied area can yield new fungal isolates with metabolites to be investigated. Further studies on the isolation and identification of fungi as well as investigating and prospecting their secondary metabolites for biotechnological uses or biologically important application, represent a critical need nowadays. Fungi isolated from medicinal plants usually exerts novel and important biological activities. Documenting the species composition of fungal mycobiota existing over the world and comparing this information may contribute in understanding relation between presence of such genera and many other factors.

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