BIORESEARCH BULLETIN
Sapience Research Labs

ORIGINAL PAPER OPEN ACCESS

Biodiversity of Soil Fungi: Why, how and where?

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Received: 20 November 2010 / **Accepted:** 14 December 2010 / **Published online:** 02 February 2011

Abstract

Fungal biodiversity studies have gained momentum in the last three decades with the primary objective of documenting the astounding numbers of fungi occurring in nature. Available literature reveals that majority of the fungi still await discovery. With quantum changes in the frontier areas of the life sciences, biodiversity studies have undergone many changes with regard to methodology adopted for identification of fungal species. Biochemical and molecular methods have replaced traditional ones. Understanding community dynamics on fungi is as important as identification and nomenclature of fungal species. Fungal diversity studies have been undertaken in different parts of the globe; yet many regions continue to remain unexplored. This article emphasizes the urgency to make use of the modern tools of science to unravel the fungal biodiversity in such unexplored regions. This would simultaneously provide thrust to the cataloguing of fungal species and documenting the patterns of fungal communities, which would further have added value from the ecological and biotechnological perspective.

Key Words: Biodiversity, Soil fungi, Fungal ecology, Fungal systematic, Community profiling.

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Article citation:
Vishwanathan AS. 2011. Biodiversity of Soil Fungi: Why, how and where?. Bioresearch Bulletin 4: 205-211.

INTRODUCTION

It was about halfway through the 20th century that studies on diversity of fungi in soil, habitats of individual fungal species and fungal communities, their specific role in the biochemical processes taking place in the soil and fungal pathology gathered importance in the scientific community. Ever since, fungal diversity and soil myco-ecology have always made an interesting study for a wide range of disciplines ranging from systematics to genetic engineering.

Biodiversity studies include number and species richness of the concerned taxa (Ovreas, 2000), genetic diversity (Nannipieri et al, 2003), community structure and functional diversity. Though scientists have been amassing data about fungal diversity, they have hardly been able to comprehend how exactly the species, community and genetic diversity influence ecosystem function.

Given the advancements in methods for isolation and identification of fungal species, structuring and classification of fungal communities and assessment of fungal diversity, it becomes all the more important to know why it is necessary to study fungal diversity, how to go about it and the which are the geographical regions that need to be explored.

Need for Fungal Biodiversity Studies

Role of fungi

Fungi are known to carry out almost all biological functions to mediate up to 90 percent of the processes in the soil, along with soil bacteria (Nannipieri et al, 2003). They perform important functions in relation to soil structuring, water dynamics, nutrient cycling, breaking down of complex organic matter into simpler molecules for ready uptake by plants, enhancing assimilation and absorption in plants by mutual association, disease suppression in plants and modification of allelopathic activity of plants and a multitude of other ecologically important functions (Inderjit, 2005;Jenkins, 2005;Sumbali, 2005). Moving on to a more tangible perspective, fungi play a significant role in the daily life of human beings, encompassing a wide spectrum of themes ranging from agriculture to medicine and industry to environmental remediation (Manoharachary et al, 2005;Wainwright, 1992). Fungi fulfill a range of important ecological and social functions, yet current understanding of fungal biodiversity in soil is limited (Anderson and Cairney, 2004;Hawksworth and Rossman, 1997;Kennedy and Gewin, 1997).

The number game

Fungi are among few of the most diverse organism groups on this planet which have been inadequately explored and studied (Hammond, 1995). Hawksworth (2002) is of the firm opinion that more detailed studies, especially in particular sites in the tropics, are needed to make a confident overall estimate of the fungal numbers. Around one -third of the fungal diversity of the globe is believed to exist in India, referred to as 'the cradle for diverse groups of fungi' (Manoharachary et al, 2005).

Fungi are represented by a magnitude of species, speculated to be next in number only to insects (Hawksworth, 1991). The actual extent of fungal diversity in the soil is much greater than we can possibly estimate at the moment. By the end of the previous century, only about 70,000 of the estimated 1.5 million fungal species had been isolated, identified and characterized (Hawksworth and Rossman, 1997). Quite a large proportion of the fungal population of the world continues to elude the scientific community. Jones & Bradford (2001) state that species richness coupled with poor taxonomic studies have led to there being no complete inventory of soil biota being available for any habitat or region.

Fungal systematics

Fungal systematics, in the context of these ecological and economic entailments, assumes far greater importance in biological research. Fulfillment of the needs of diverse taxonomic users requires that a system of classification be developed using modern tools of science. Fungal systematics involves identification of the unknown fungus, determination of its relationships with known fungi, nomenclature and classification (Shenoy et al, 2007). Classification includes description, storage of information and prediction of phylogenetic relationships of the organism (Judd et al, 2002).

Functional diversity

Mere assessment of community composition hardly provides any information about functions of microbes in soils (Leckie, 2005). Zak & Visser (1996) stress on the need to study the functional attributes of fungi in an ecosystem in addition to their taxonomic characteristics. A precise understanding of the functional characteristics of microbial communities in soil is necessary to comprehend key ecological processes (Lalor et al, 2007).

Techniques for Studying Fungal Diversity

Primary methods

Current knowledge of the diversity of soil fungi is primarily based on direct observation or cultures obtained from soil isolation exercises. Fungal species that exist as mycelia cannot be observed by direct observation and those fungal spores which are unable to grow and sporulate in culture media continue to remain obscure to the scientific community.

The traditional method of random soil sampling could lead to a gross under-estimation of the microbial diversity and population size, leading to high variability among replicates and low statistical resolution (Klironomos et al, 1999). Soil sampling must be done on a smaller scale with more number of samples for estimating microorganisms in the soil microhabitat (Grundmann and Gourbiere, 1999).

Unlike bacteria, not many fungi can be cultured by standard laboratory methods (Thorn, 1997). Soil plating and direct viable count methods have been employed traditionally in the estimation of soil biodiversity. These techniques, though rapid and inexpensive, have their disadvantages in terms of comprehensiveness of results, suitable for only those species which sporulate extensively (Tabacchioni et al, 2000). Culture media are rich sources of carbon as compared to the substrates encountered in situ and species growing faster may be at an advantage thereby bringing in some discrepancies in the cultured community composition (Nocker et al, 2007). However, soil plating methods can be used to provide a superficial estimate of the dominant species in a given region.

A high level of morphological variability often leads to confusion in identification and nomenclature especially when the identification characteristics are affected (Ortega et al, 2008). Obtrusive issues, such as the need for different procedures for detection and identification of fungi, make the process of nomenclature and taxonomy more complicated (Hawksworth, 2004). A major bottleneck is the inability to describe fungal species with accuracy by means of only their sexual state (Horton, 2002). As a result, systematics is now being supplemented by other biochemical, molecular, immunological methods.

Advanced techniques

Though morphological characters help in identifying fungi by means of descriptions and identification keys, they are not very effective in terms of determination of phylogenetic relationships (Judd et al, 2002). Recent advances in DNA sequence technologies and analytical methods have revolutionised fungal systematics. Emphasis has shifted from culturing methods to analysis and assessment of signature molecules. Molecular characters such as DNA sequence-data are essentially advantageous as they offer a greater number of discrete characters, which can be analysed statistically to infer phylogenetic relationships (Shenoy et al, 2007). These tools have become an almost indispensible part of fungal systematics.

Species identification and classification by morphological methods can be supplemented by terminal restriction fragment length polymorphism (T-RFLP) analysis of the Internal Transcribed Spacer (ITS) sequences (Ortega et al, 2008); (Cui et al, 2008); membrane-based ITS macroarrays coupled with community ITS probes (Izzo and Mazzola, 2009); fluorescein diacetate (FDA) hydrolysis, single carbon source substrate utilization (SU) profiles and fatty acid methyl ester (FAME) profiles (Larkin, 2003); 18S rRNA profiles (Bastias et al, 2007); fungal-specific PCR of soil DNA coupled with denaturing gradient gel electrophoresis (DGGE) (Oros-Sichler et al, 2006); EF1-α nucleotide sequences (Alves et al, 2008); GC content analysis (Nusslein and Tiedje, 1999) and DNA-DNA hybridization (Greene and Voordouw, 2003).

A novel DNA barcoding system for fungal identification at the species level using mitochondrial *cytochrome c oxidase 1 (CO1)* sequences (Seifert et al, 2007); *COX1*, ITS and D1/ D2 sequences (Letourneau et al, 2010) and multiple loci (Roe et al, 2010) have been reported. Phylogenetic analyses are then performed using different methodologies based on nuclear ribosomal DNA sequences, 5.8S gene region (Huang et al, 2009); ITS sequences (Nilsson et al, 2008) and protein-coding gene sequences (Tang et al, 2009) followed by application of different sequence analysis methods (Peláez et al, 2008).

Analysis of genetic and taxonomic diversity of soil microbes is quite a time consuming and laborious process (Lalor et al, 2007). Though many molecular tools are being employed to structure microbial communities and assess fungal diversity, further refinement is necessary to use these methods for identification of individual fungal species. Moreover, these methods may not be very effective

until reference genome sequences are available, in the public domain, for majority of the fungal species in the soil. Interestingly, it has also been found that in some of the cases, the protocols adopted tend to affect the molecular parameters being studied, resulting in discrepant results (Kirk et al, 2004).

Kennedy and Clipson (2004), Leckie (2005) and Nocker (2007) are comprehensive reviews, written in recent years, on the microbial community profiling methods in vogue. Hyde and Soytong (2007) (Hyde and Soytong, 2007) provide a critical evaluation of the advances in microfungal diversity.

Fungal Diversity Studies Around The Globe

Hyde (2002) points to the occurrence of a large number of undescribed fungal species which 'may occur in poorly studied countries, hosts, habitats, niches or tissues, and are mostly microfungi'. Research in low biodiversity, extreme environments should be applicable to understanding soil biodiversity in more complex, temperate and tropical ecosystems. Wall & Virginia (1999) opine that research in such regions may provide information on the response of soil biodiversity to increasing occurrences of extreme climates predicted to occur from the global change models.

An extraordinary number of genera and species of fungi, including many new taxa, have been isolated from soils of varied habitats: from barren desert sands to forest soils rich in humus. There are extensive reports and publications on biodiversity studies of soil fungi carried out in different parts of the world – from the dense forests of the Western Ghats (Satish et al, 2007) and deciduous forest soils (Vishwanathan, 2010) of India to the hilly terrains of Israel (Grishkan and Nevo, 2008) and from damp and musty caves in Puerto-Rico (Nieves-Rivera, 2003) to the icy plains of the Antarctic (Ruisi et al, 2007).

CONCLUSION

Microbial biodiversity studies are essential for basic scientific research as well as to gain more insights into community structure and function. Scientific studies utilize the naturally available fungal resources to optimum levels in order to enhance the standard of life as it stands today. Biodiversity studies will have to encompass molecular, genetic and ecological factors in order to get a well-defined picture of the situation.

Having understood the advantages and limitations of the different traditional, biochemical and molecular methods of estimating fungal diversity, it becomes necessary to develop more efficient, simplified-yet-high-resolution protocols which can provide a realistic estimate of microbial community dynamics. However, a caveat to be noted is that any method chosen must be critically evaluated before acceptance.

Cannon (1997) enlists some of the reasons for inadequate studies in fungal biodiversity to be the lack of trained staff and appropriate identification manuals and more importantly the occurrence of large numbers of fungal species in small study sites, only a small proportion of which have been adequately characterized. Another important point mentioned herein, that needs to be highlighted, is the inconspicuous nature of many fungal species and the insufficient attention given to systemic mycology by funding agencies as the other shortcomings in this regard.

Fungal diversity studies in different ecosystems are the need of the hour in order to get a clear picture of the biodiversity. Though this article focuses primarily on soil fungi, there is equal if not greater scope for evaluating diversity of fungi in other environments as well. Fungal systematics is a complicated science, yet it is a basic necessity.

ACKNOWLEDGEMENTS

The author expresses gratitude to the Revered Chancellor of Sri Sathya Sai Institute of Higher Learning, Bhagawan Sri Sathya Sai Baba, for His constant guidance and support.

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