

# 1 Detection and Diversity of Fungi from Environmental Samples: Traditional Versus Molecular Approaches

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## 1.1 Introduction

Microbial life within the soil ecosystem is a fascinating aspect of soil biology, and has recently caught the attention of microbiologists. Many fungi grow in the soil and some have evolved to thrive in harsh conditions, such as those found in acidic or alkaline soils. These microorganisms can be considered as “highly developed” as they flourish and reproduce in these ecological niches and unusual habitats and have successfully made use of soil and its nutrients for their energy sources. Fungi are an important component of the soil microbiota, they mediate important ecological processes such as nutrient recycling, and they maintain important symbiotic relationships with plants and bacteria (Garrett 1981; Parkinson 1983; Yu et al. 2005). Many fungi are pathogenic (e.g. Jaworski et al. 1978; Cahill and Mohr 2004) and some may be useful in bio-exploitation (e.g. Vinokurova et al. 2003). The realms of soil mycota are possibly the largest on the planet.

A diverse range of fungi are present in soil ecosystems and include ascomycetes, basidiomycetes, some being ectomycorrhizal fungi, anamorphic fungi and arbuscular mycorrhizal fungi (AMF). At present, there is no clear morphological, phylogenetic or ecological definition of soil fungi. Any definitions based on these concepts are very difficult to implement because the soil ecosystem harbours a plethora of fungi with great morphological, genetic and functional diversity and lacks geographic boundaries. Perhaps the best definition of soil fungi should be encapsulated in the word itself (fungi from soil!). Most of our current knowledge of soil mycota is based on traditional systematics, which does not reflect any real sense of evolutionary relationships. The interaction between

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these fungi with plant roots and other biotic or abiotic factors within the soil constitutes a challenge to soil microbiologists. Obviously there must have been a long evolutionary history of adaptation and competition that permitted fungi to evolve in diverse forms and interact with other organisms.

In this chapter we explore the limits of conventional and molecular techniques used to assess and detect soil microfungi diversity and provide insights into their feasibility. In particular we address the problems associated with the dilution plating technique, importance of the rDNA gene in fungal systematics, the reliability of other molecular approaches (especially denaturing gradient gel electrophoresis; DGGE) and their drawbacks.

## 1.2

### Microscopy and Culture-Based Methods

Traditional methods to assess fungal diversity in soil environment rely mainly on the dilution-plating technique (coupled with the use of selective media) and microscopy to identify sporulating fungal bodies. Davet and Rouxel (1997) have already detailed all the experimental procedures commonly used in the dilution plate method and direct comparison. Both methods are direct isolation techniques; and the dilution-plating method involves a combination of gentle dispersion, soil dilution and serial dilution, small amounts of which are ultimately plated on artificial media and incubated. The direct comparison method involves sprinkling of a known amount of soil onto a medium, which is then incubated (Davet and Rouxel 1997). Both methods provide a reasonably sensitive recognition of soil fungi and have been widely used in diversity studies in different habitats (e.g. Elmholt et al. 1999; Cho et al. 2001; Cabello and Arambarri 2002). Cultural methods, coupled with morphological details from microscopy, are among the earliest techniques used and allow one to detect exactly which taxon is present (identification). They have also commonly been used because of their simplicity, low cost and the fact that they are easy to conduct. Williams et al. (1965) has already detailed the efficiency of the soil washing technique, its applicability and potential for studying soil microhabitats and these are not detailed here. While these methodologies are easy, fast and reliable in finding the dominant culturable fungal taxa, they have a number of limitations which impede a proper diversity assessment.

Davet and Rouxel (1997) mentioned that the traditional methods outlined above tend to overestimate species that sporulate in soil, while those in mycelial state or those that have slow growth in culture are largely overlooked. In addition, most of these methods result in isolation of only the most common and abundant fungi (often referred to as “generalists”), such as the asexual ascomycetes *Fusarium*, *Penicillium* and *Trichoderma* and oomycetes (*Pythium*). These cultivated organisms are those that can utilise the energy source under

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