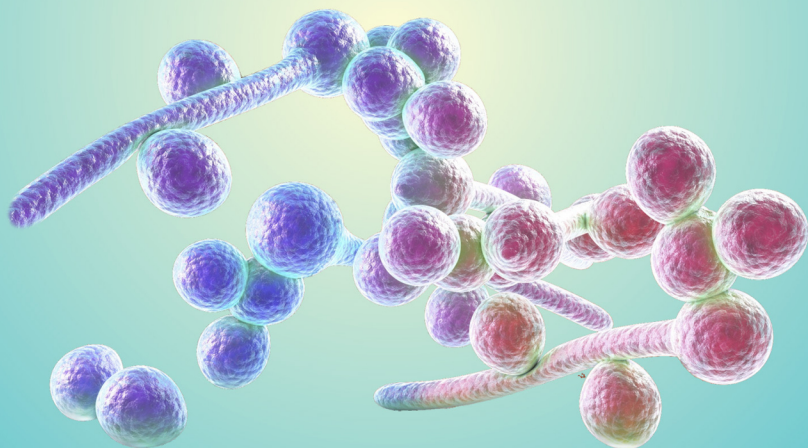


# Methods in Fungal Biology

A Manual of Laboratory Protocols





**METHODS IN FUNGAL BIOLOGY:  
A MANUAL OF LABORATORY PROTOCOLS**

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# **METHODS IN FUNGAL BIOLOGY: A MANUAL OF LABORATORY PROTOCOLS**

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## **PREFACE**

Fungi are one of the key components in biosphere as they play an essential role in recycling of nutrients in all terrestrial habitats. In nutrient cycling, they perform as decomposers, parasites and symbionts. Interestingly, fungi are ubiquitous in nature and present everywhere around us. The study of fungi in various habitats, their relation with respective substrates and environment is known as mycology. Despite studying their important roles in the biosphere, it is also important to explore all categories of fungi. The practice of mycological studies in present era is connecting a bridge between the concepts of mycologists of older era with modern concepts of fungi. These step by step systematic developments in mycology are now providing new directions and dimensions to this field. To keep this pace ever progressing, mycologists should to be focused on methods and protocols, which is the integral part of any biological studies. To achieve this, creation and development of innovative mycological teaching, research and extension aids are required. Improved and more extensive text books, laboratory manuals or hand books that provide the recent and suitable techniques are some essentials which enable students, researchers and scientists to understand all aspects of fungi, so that these can be processed accordingly. This book is a sincere attempt in this regard.

Although, sufficient literature on mycological methods is available in different journals, periodicals, etc., if some need any mycological information in time, it is not possible to collect it from scattered literature. It is also not possible for any institution to have all literature readily. Additionally, variation in single technique described by many scientists has been observed which creates confusion among students, researchers and scientists. Therefore, this book is designed to provide detailed informations on methods in fungal biology especially isolation and identification.

This book comprises of three parts that eventually deals with the broad spectrum of the methods, techniques, instrumentations, stains, cultures and reagents used in mycological studies. First part (PART - A) contains information about instrumentation, techniques, stains, cultures and reagents. The detailed information on type of the instrument, its principle, working and even precautions to be taken during handling is described here. The information on type of culture media, stains and reagents is also provided. The second part (PART - B) describes about fungal isolation and identification. The protocols used for the isolation of fungi from various substrates as endophytes, lichenicolous fungi, keratinophilic fungi, mycorrhizal fungi and as plant pathogens are separately mentioned. The characters used for identification of each types of fungi are also dealt. Identification keys of fungi are covered and compiled in this book. In third part (PART - C), methods of long term preservation of fungi are discussed in detail. The safety norms and regulations in handling fungal specimens along with appendix to stains, cultures and reagents are also provided here. Drawing together coloured pictures have made this work a unique document in mycology and a pivotal contribution to this field.

The proposed book is unique of its kind as it provides complete basic information on mycology for readers at one place instead of searching for scattered information. The contents covered are described in detail in simple and understandable language. Each method used in fungal biology are discussed in simpler way to make it reader friendly, and is useful for students, researchers and scientist especially the beginners.

The authors appreciate receiving suggestions, remarks and constructive criticism (a2gautam2006@gmail.com) that would improve the quality of the book.

**Ajay K. Gautam**

**Shubhi Avasthi**



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**Ajay K. Gautam**

**Shubhi Avasthi**

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## INTRODUCTION

The kingdom fungi includes some of the interesting ecologically and economically important organisms which are heterotrophic in nature. They are unable to manufacture their own food as photosynthetic organisms do. Most of the fungal species are saprophytic. By breaking down dead organic material, they continue the cycle of nutrients through ecosystems. They are however, parasitic and mutualistic too. While inhabiting parasitic mode, fungi cause a number of plant diseases whereas, in mutualistic approach they occupy plants as symbiotic association. Most of the vascular plants could not grow without the symbiotic fungi, or mycorrhizae that inhabit their roots and supply essential nutrients and or endophytes that provide resistance to plant against pathogens, salt and drought. Plant parasites on the other hand causes severe damage to infected host morphologically, physiologically and biochemically. The plant diseases caused by fungi include rusts, smuts, mildews, necrosis, sooty moulds and rots may cause severe damage. However, a number of fungi, in particular the yeasts, are important “model organisms” for studying problems in genetics and molecular biology.

Fungi can break down most of the organic compounds including lignin, a major component of wood which is difficult to break down easily. They secrete extracellular enzyme in the plant host to draw nutrients and also store as glycogen. These patterns of nutritional uptake differentiate fungi as saprophytic, parasitic and symbiotic. This variation not only alters their morphological, physiological and ecological characteristics, but also leads to adapt separate and specific method for isolation and identification of saprophytic, parasitic and symbiotic fungi.

The saprophytic fungi decay dead organic materials present anywhere on earth i.e. soil, air and even water. These fungi inhabit dead plant and animal parts, help in their decay and release organic nutrients to the environment. The plant litter including wooden logs, leaves, branches and barks are used by fungi as food drawing sources. Similarly, parasitic fungi also inhabit plant parts but the living tissues only. They arrest physiological and nutritional mechanisms of plant hosts and utilize them for their own growth and development and ultimately harm the plant with reduced growth or even death. In symbiotic mode, the fungi establish a mutual relationship with the host. Here both partners i.e. fungi and plant are in equal benefit, as plant provides shelter and photosynthetic products for fungus and in return fungi absorb nutrients for the plant from soil and environment. As all saprophytic, parasitic and mutualistic fungi have their morphological, microscopic, physiological, biochemical and genomic characteristics which are useful in taxonomic description.

The kingdom fungi embrace diversity of fungal life forms in various taxa with varied ecologies, life cycle strategies. It has been estimated that around 1.5 million species of fungi are existing on earth of which about 5% has been formally classified (Gupta and Paul 2004; Mahaharachary 2005; Dix and Webster 1995; Agrios 2005; Katoch and Kapoor 2014). For taxonomical evaluation of fungi, the correct identification is most important factor among all. The basic concepts used for identification include conventional phenotypic methods based on morphological and microscopic phenotypic characteristics of fungi. However, conventional phenotypic methods are often time-consuming and laborious. The influence of culture conditions on unstable and subjective nature of phenotypic characteristics also point out limitation of phenotypic methods. The fungi that do not grow in culture can also not be identified with conventional methods. The molecular approach on the other hand, involves the use of genes to gain taxonomic status of organisms and their evolutionary relationships have influenced inducing significant changes in the traditional concepts of systematics. The molecular systematics

have provided new information in fungal systematics by using different techniques like RFLP, RAPD, rDNA analysis, SSR and ISSR and helped in reshaping the classification of Kingdom Fungi (Gherbawy and Voigt 2010; Raja et al. 2017).

Therefore, the present compilation is an effort to provide all important information on isolation and identification of fungi including their ecological habitats and interactions with host/substrates.

