Fungal Biology

Vijai Kumar Gupta · Maria G. Tuohy
Editors
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Associate Editors

Laboratory Protocols in Fungal Biology

Current Methods in Fungal Biology

Springer

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Foreword

Fungi represent the fifth kingdom of organisms, which is characterized—second only to prokaryotes—by a huge number of diverse species. Even more, fungi have developed a tremendous variety in lifestyles, biochemical properties, and morphological characters, the latter having been a permanent challenge for defining species and their identification. They have conquered practically all habitats, from deep sea water to desert soil, and from prokaryotes to mammals, leading to an array of positive but also negative impacts on mankind. On the negative side, fungi are known as pathogens of plants—a situation which seriously affects crop plantations all around the earth—but also of higher fungi, of lower eukaryotes, and of all animals up to mammals and men. Also, their versatile metabolism provided them with efficient abilities to colonize almost all material, leading to biodeterioration of various organic materials including paintings and covers, which allowed them to settle in buildings and flats resulting in indoor contamination as a major problem of today. Yet there are also numerous benefits: many fungi are known as beneficial symbionts of plants, such as plant tissue endosymbionts and mycorrhizas. In fact, the earth would be devoid of plants in the absence of the latter. Finally several fungi have been domesticated by humans, either for their use in agriculture (such as for biocontrol of plant or invertebrate pathogens or in plant growth protection and stimulation), for the preparation of feed- and foodstuff, and as efficient producers of biotechnological products such as primary metabolites, numerous enzymes, and antibiotics. In the area of modern molecular biotechnology, fungi such as *Pichia pastoris* have become important high-throughput hosts for the production of recombinant proteins of bacterial to human origin. Last but not least, fungi like *Saccharomyces cerevisiae, Neurospora crassa*, and *Aspergillus nidulans* have become model systems for basic biochemical and genetic research, and an impressing amount of our textbook knowledge would not be available without them. In the current genomic age, elucidation of the genome inventory of about 50 multicellular asco- and basidiomycetes and the same number of yeasts has been completed and opened new avenues for their investigation.

In view of this steadily increased interest in fungi, also the methods needed for their isolation and identification, as well as their genetic manipulation and monitoring of gene expression and protein production, have become refined and complemented. This book aims at presenting an inventory of techniques and methods that are currently in use for studying fungi: it contains 57 chapters dedicated to description of these techniques, starting from concepts of

cultivation, enumeration, and visualization of fungi; molecular approaches for detection and quantification; measurement of relevant enzymes and methods for their application; and the use of bioinformatic tools to investigate fungal genomes.

As a professional reference, this book is aimed at all people who work with fungi and should be useful both to academic institutions and research teams, as well as to teachers, graduate and postgraduate students.

Vienna, Austria Prof. Christian P. Kubicek

Foreword

It gives me immense pleasure to write a foreword for *Laboratory Protocols in Fungal Biology* of Springer, USA edited by Dr. Vijai Kumar Gupta and Dr. Maria G. Tuohy. After going through the content of this laboratory protocol, I feel that it is a wonderful attempt done by Dr. Gupta to compile together all the information about the subject that will be highly useful to all mycologists around the globe. I am sure that this volume will be highly useful to all those concerned with fungi and their biology, including environmental and public health officers and professionals in the field of interest. The volume is really exhaustive covering almost all the aspects of fungal biology. It will also be of interest to postgraduate students in this field and also for one and all interested in Fungi. Additionally it will be of great market value. This effort of Dr. Gupta's is admirable.

Varanasi, India — Prof. R.S. Upadhyay

Preface

The interaction between fungi and their environment is central to many natural processes that occur in the biosphere. The hosts and habitats of these eukaryotic microorganisms are very diverse; fungi are present in every ecosystem on Earth. The fungal kingdom is equally diverse, consisting of seven different known phyla. Yet detailed knowledge is limited to relatively few species. The relationship between fungi and humans has been characterized by the juxtaposed viewpoints of fungi as infectious agents of much dread and their exploitation as highly versatile systems for a range of economically important biotechnological applications. Understanding the biology of different fungi in diverse ecosystems as well as their interactions with living and nonliving is essential to underpin effective and innovative technological developments.

The tools and techniques of molecular biology, once reserved for mammalian and bacterial systems, have been adapted and optimized for the analysis of fungal species at the molecular level. Rapid screening techniques based on screening specific regions in the DNA of fungi have been used in species comparison and identification and are now being extended across fungal phyla with the ultimate goal being the assembly of the "Fungal Tree of Life" by the US National Science Foundation. Within a decade after the Human Genome Sequence was published, genome sequencing technology has been adapted to yield the complete genome sequences of not only fungi of commerce and medical relevance, but other more isoteric species. Post-genomics approaches and systems biology are now also being applied to understanding the details of fungal biology and the interactions between fungi, their hosts, and their environment. The majority of fungi are multicellular eukaryotic systems and therefore may be excellent model systems by which to answer fundamental biological questions. A greater understanding of the cell biology of these versatile eukaryotes will underpin efforts to engineer (e.g., "humanize") certain fungal species to provide novel cell factories for production of proteins for pharmaceutical applications. Finally, renewed interest in all aspects of the biology and biotechnology of fungi may also enable the development of "one pot" microbial cell factories to meet consumer energy needs into the twenty first century. To realize this potential and to truly understand the diversity and biology of these eukaryotes, continued development of scientific tools and techniques is essential.

This publication aims to provide a detailed compendium of analytical methods used to investigate different aspects of mycology, including fungal

biology and biochemistry, genetics, phylogenetics, genomics, proteomics, molecular enzymology, and biotechnological applications, in a manner that reflects the many recent developments of relevance to scientists investigating the Kingdom of Fungi.

Galway, Ireland

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Safety Norms and Regulations in Handling Fungal Specimens

1

Finola E. Cliffe

Abstract

This chapter provides basic safety information required when handling fungal cultures and when performing the procedures outlined in this manual. Several topics are discussed including routine precautions when working with fungal organisms.

Keywords

Fungi • Mycology • Health and safety • Biosafety • Biosafety levels

Introduction

Biosafety measures designed to ensure the safety of laboratory workers include the use of various primary and secondary barriers, many of which are due to the advent of new technologies in the fields of materials science and engineering. Personnel undertaking the protocols in this manual may come across potentially hazardous materials such as pathogenic and infectious biological fungal agents, in addition to toxic chemicals and carcinogenic, mutagenic, or teratogenic reagents. In the case of fungal specimens, it has long been acknowledged that laboratory workers can attain infections from the agents they work with.

There have been many reported cases of laboratory-acquired infection, with countless more cases undoubtedly left unreported. Inhalation appears to be the most prominent route of exposure. Fungal hyphae in nature develop structures such as conidia on fruiting bodies or hyphal elements that develop into transmissible subsegments, which are ultimately designed for optimum dispersal in air. These elements are designed to be readily discharged, resistant to desiccation, and to remain aloft for long periods of time. Once inhaled by a host, the conidia develop into the yeast phase and can be found in the tissue of infected hosts [1]. Even with the advances in biosafety training and education, laboratory-acquired

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fungal infections continue to occur. The dimorphic fungi *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Histoplamsa caspsulatum*, for example, were found to be responsible for the majority of laboratory-acquired fungal infections in the United States [2–4]. Laboratory-associated pulmonary infections have occurred following the inhalation of conidia from mold-form cultures of *B. dermatitidis* [5, 6] and local infections from the accidental parenteral inoculation with infected tissues or cultures containing yeast forms of the fungus [7, 8] have been documented. Various reports of laboratory-associated *C. immitis* are reported in the literature prior to 1980 [9–11] including a case recorded by Nabarro [12] where a biochemist developed an intense acute infection after working with a colonial growth. Laboratory-associated histoplasmosis occurs mainly through inhalation of conidia produced by the mold form of the fungus [4, 13]; however, cutaneous infections have occurred due to accidental inoculation [14, 15].

These incidences indicate the ongoing occurrences of laboratory-acquired infections as a result of simple and preventable laboratory errors. As mentioned, the bulk of laboratory-acquired fungal infections are caused by inhalation of infectious conidia from the mold form, resulting in pulmonary infections; for example, the simple processes of opening of a culture plate lid can result in the release of large numbers of conidia [16]. To reduce the risk of infection it is practical to handle all fungal cultures under the conditions of biosafety laboratory containment BSL-2 or BSL-3 [17].

New biosafety technologies and associated guidelines have been developed to considerably improve ways to safely use fungal material. An enhanced understanding of the risks associated with various manipulations of many agents transmissible by different routes has enabled laboratory workers to apply appropriate biosafety practices to specific laboratory areas. These safety guidelines include engineering controls, management policies, work practices and procedures, as well as medical interventions. However, users must always progress with the caution associated with good laboratory practice, under the supervision of personnel responsible for implementing laboratory safety programs at their institutions.

Biosafety Levels

Several biosafety levels (BSL) have been developed for laboratories to provide increasing levels of staff and environmental protection. BSLs are guidelines that describe appropriate containment equipment, facilities, and procedures for use by laboratory workers. The BSLs range from biosafety level 1 (BSL 1) to biosafety level 4 (BSL 4), and each BSL is based on the increased risk associated with the pathogenicity of the microorganisms encountered. Most clinical microbiology laboratories follow BSL 2 practices. When working with highly infectious agents for which the risk of aerosol transmission is greater, laboratories should follow BSL 3 practices.

BSL-1 is suitable for working with fungal agents that are not known to cause disease in healthy humans. BSL-1 practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. It is important to remember, however, that many agents not ordinarily associated with disease processes in humans are opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.

BSL-2 should be used for work involving fungal agents that pose a moderate potential hazard to laboratory workers. These agents include the large group of opportunistic fungal

pathogens such as *Aspergillus* spp. and *Fusarium* spp. Some protocols can be carried out on an open bench providing the potential for aerosol production is low [17]. Although organisms regularly employed at Biosafety Level 2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a biological safety cabinet (BSC) or safety centrifuge cups. Personal protective equipment (PPE), such as splash shields, face protection, gowns, and gloves should be used as appropriate. In addition, secondary barriers such as hand-washing sinks and waste decontamination facilities must be accessible to decrease the chance of environmental contamination [18].

BSL-3 is appropriate for work with infectious agents, which may cause serious or potentially lethal diseases as a result of inhalation. The fungal pathogens *C. immitis* and *H. capsulatum* fall into this group. Autoinoculation, ingestion, and exposure to infectious aerosols are the main hazards to personnel working with these organisms. All laboratory operations should be performed in a BSC or other enclosed apparatus, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory. Within this level, primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols have been highlighted [18].

At present, no fungal agents have been classified for use at BSL-4. BSL-4 practices, safety equipment, and facility design and construction are applicable for work with hazardous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route, and for which there is no available vaccine or therapy. The primary hazards to personnel working with Biosafety Level 4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals pose a high risk of exposure and infection to laboratory personnel, the community, and the environment. The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied, positive-pressure personnel suit. The BSL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment [18].

The safety plan of a laboratory should address general considerations, chemical safety, and section-specific safety. In the case of mycology laboratories, as with all laboratories, each section requires a site-specific risk assessment to address biohazard considerations and to outline measures for staff protection. Table 1.1 outlines an example of the type of assessment that should be performed.

Materials (See Note 1)

1. Sterile distilled water
2. PPE such as coats, gowns, gloves, masks, face shields, safety glasses
3. Ethanol (70%)
4. Biosafety cabinet
5. Eyewash station
6. Hand washing sinks
7. HEPA filtered respirators or masks
8. Plasticwear (substitute for glass)
9. Centrifuge safety cups
10. Containers for transport of specimens, waste, and sharps.
11. Biohazard bags
12. Biohazard labels
13. Automatic or mechanical pipetting devices