Edited by
Karl Esser

THE MYCOTA

A Comprehensive Treatise on Fungi
as Experimental Systems for Basic and Applied Research

Physiology and Genetics XV

Selected Basic and Applied Aspects

Timm Anke and Daniela Weber
Volume Editors

Springer

The Mycota

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K. Esser

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XV

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Applied Aspects

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Karl Esser
(born 1924) is retired Professor of General Botany and Director of the Botanical Garden at the Ruhr-Universität Bochum (Germany). His scientific work focused on basic research in classical and molecular genetics in relation to practical application. His studies were carried out mostly on fungi. Together with his collaborators he was the first to detect plasmids in higher fungi. This has led to the integration of fungal genetics in biotechnology. His scientific work was distinguished by many national and international honors, especially three honorary doctoral degrees.

Timm Anke
(born 1944) studied Biochemistry at the University of Tuebingen, where he completed his PhD degree with a dissertation on the biosynthesis of fungal siderophores. In 1973 he joined Fritz Lipmann's group of at the Rockefeller University in New York City, where he investigated the biosynthesis of valinomycin, a streptomycete ionophore. After his return to Tuebingen in 1975 he started organizing a group searching for new antibiotics from basidiomycetes within the framework of Hans Zaehner's Collaboratorive Research Center (SFB 76) focusing on the chemistry and biology of microorganisms. In 1981 he became full Professor of Biotechnology at the University of Kaiserslautern and since 1998 he has headed the Institute of Biotechnology and Drug Research (IBWF e. V.) in Kaiserslautern. One of his outstanding achievements in the field of antibiotic research is the discovery of the strobilurins, a major class of agricultural fungicides, for which he was awarded the Karl-Heinz-Beckurts Prize in 1996.

Daniela Weber
(born 1978) studied natural products from endophytic fungi during her graduate work at the University of Kaiserslautern's Department of Biotechnology. She received a Chemiefonds fellowship for Ph.D. students. Her Ph.D., completed in 2006, focused on the isolation of endophytic fungi from medicinal plants and the investigation of the fungal metabolites. She later continued her work at the Institute of Biotechnology and Drug Research (IBWF, Kasiserslautern). In 2008 she accepted a position at MIP International Pharma Research GmbH (St. Ingbert, Germany). Her fields of activities are pharmaco-toxicological and clinical expert reports, pharmacovigilance, and regulatory affairs.

Series Preface

Mycology, the study of fungi, originated as a sub discipline of botany and was a descriptive discipline, largely neglected as an experimental science until the early years of this century. A seminal paper by Blakeslee in 1904 provided evidence for self incompatibility, termed "heterothallism", and stimulated interest in studies related to the control of sexual reproduction in fungi by mating-type specificities. Soon to follow was the demonstration that sexually reproducing fungi exhibit Mendelian inheritance and that it was possible to conduct formal genetic analysis with fungi. The names Burgeff, Kniep and Lindegren are all associated with this early period of fungal genetics research.

These studies and the discovery of penicillin by Fleming, who shared a Nobel Prize in 1945, provided further impetus for experimental research with fungi. Thus began a period of interest in mutation induction and analysis of mutants for biochemical traits. Such fundamental research, conducted largely with Neurospora crassa, led to the one gene: one enzyme hypothesis and to a second Nobel Prize for fungal research awarded to Beadle and Tatum in 1958. Fundamental research in biochemical genetics was extended to other fungi, especially to Saccharomyces cerevisiae, and by the mid-1960s fungal systems were much favored for studies in eukaryotic molecular biology and were soon able to compete with bacterial systems in the molecular arena.

The experimental achievements in research on the genetics and molecular biology of fungi have benefited more generally studies in the related fields of fungal biochemistry, plant pathology, medical mycology, and systematics. Today, there is much interest in the genetic manipulation of fungi for applied research. This current interest in biotechnical genetics has been augmented by the development of DNA-mediated transformation systems in fungi and by an understanding of gene expression and regulation at the molecular level. Applied research initiatives involving fungi extend broadly to areas of interest not only to industry but to agricultural and environmental sciences as well.

It is this burgeoning interest in fungi as experimental systems for applied as well as basic research that has prompted publication of this series of books under the title The Mycota. This title knowingly relegates fungi into a separate realm, distinct from that of either plants, animals, or protozoa. For consistency throughout this Series of Volumes the names adopted for major groups of fungi (representative genera in parentheses) areas follows:

Pseudomycota

Division:	Oomycota (Achlya, Phytophthora, Pythium)
Division:	Hyphochytriomycota

Eumycota

Division:	Chytridiomycota (Allomyces)
Division:	Zygomycota (Mucor, Phycomyces, Blakeslea)

Division:	Dikaryomycota
Subdivision:	Ascomycotina
Class:	Saccharomycetes (Saccharomyces, Schizosaccharomyces)
Class:	Ascomycetes (Neurospora, Podospora, Aspergillus)
Subdivision:	Basidiomycotina
Class:	Heterobasidiomycetes (Ustilago, Tremella)
Class:	Homobasidiomycetes (Schizophyllum, Coprinus)

We have made the decision to exclude from The Mycota the slime molds which, although they have traditional and strong ties to mycology, truly represent nonfungal forms insofar as they ingest nutrients by phagocytosis, lack a cell wall during the assimilative phase, and clearly show affinities with certain protozoan taxa.

The Series throughout will address three basic questions: what are the fungi, what dothey do, and what is their relevance to human affairs? Such a focused and comprehensive treatment of the fungi is long overdue in the opinion of the editors.

A volume devoted to systematics would ordinarily have been the first to appear in this Series. However, the scope of such a volume, coupled with the need to give serious and sustained consideration to any reclassification of major fungal groups, has delayed early publication. We wish, however, to provide a preamble on the nature off ungi, to acquaint readers who are unfamiliar with fungi with certain characteristics that are representative of these organisms and which make them attractive subjects for experimentation.

The fungi represent a heterogeneous assemblage of eukaryotic microorganisms. Fungal metabolism is characteristically heterotrophic or assimilative for organic carbon and some nonelemental source of nitrogen. Fungal cells characteristically imbibe or absorb, rather thaningest, nutrients and they have rigid cellwalls. The vast majority of fungi are haploid organisms reproducing either sexually or asexually through spores. The spore forms and details on their method of production have been used to delineate most fungal taxa. Although there is amultitude of spore forms, fungal spores are basically only of two types: (i) asexual spores are formed following mitosis (mitospores) and culminate vegetative growth, and (ii) sexual spores are formed following meiosis (meiospores) and are borne in or upon specialized generative structures, the latter frequently clustered in a fruit body. The vegetative forms of fungi are either unicellular, yeasts are an example, or hyphal; the latter may be branched to form an extensive mycelium.

Regardless of these details, it is the accessibility of spores, especially the direct recovery of meiospores coupled with extended vegetative haploidy, that have made fungi especially attractive as objects for experimental research.

The ability of fungi, especially the saprobic fungi, to absorb and grow on rather simple and defined substrates and to convert these substances, not only into essential metabolites but into important secondary metabolites, is also noteworthy. The metabolic capacities of fungi have attracted much interest in natural products chemistry and in the production of antibiotics and other bioactive compounds. Fungi, especially yeasts, are important in fermentation processes. Other fungi are important in the production of enzymes, citric acid and other organic compounds as well as in the fermentation of foods.

Fungi have invaded every conceivable ecological niche. Saprobic forms abound, especially in the decay of organic debris. Pathogenic forms exist with both plant and animal hosts. Fungi even grow on other fungi. They are found in aquatic as well as soil environments, and their spores may pollute the air. Some are edible; others are

poisonous. Many are variously associated with plants as copartners in the formation of lichens and mycorrhizae, as symbiotic endophytes or as overt pathogens. Association with animal systems varies; examples include the predaceous fungi that trap nematodes, the micro fungi that grow in the anaerobic environment of the rumen, the many insect associated fungi and the medically important pathogens afflicting humans. Yes, fungi are ubiquitous and important.

There are many fungi, conservative estimates are in the order of 100,000 species, and there are many ways to study them, from descriptive accounts of organisms found in nature to laboratory experimentation at the cellular and molecular level. All such studies expand our knowledge of fungi and of fungal processes and improve our ability to utilize and to control fungi for the benefit of humankind.

We have invited leading research specialists in the field of mycology to contributeto this Series. We are especially indebted and grateful for the initiative and leadership shown by the Volume Editors in selecting topics and assembling the experts. We have all been a bit ambitious in producing these Volumes on a timely basis and there in lies the possibility of mistakes and oversights in this first edition. We encourage the readership to draw our attention to any error, omission or inconsistency in this Series in order that improvements can be made in any subsequent edition.

Finally, we wish to acknowledge the willingness of Springer-Verlag to host this project, which is envisioned to require more than 5 years of effort and the publication of at least nine Volumes.

Bochum, Germany — Karl Esser
Auburn, AL, USA — Paul A. Lemke
April 1994 — *Series Editors*

It is Time to Retire

During the Fourth International Mycological Congress in Regensburg (1989) while relaxing in a beer garden with Paul Lemke (USA), Dr. Czeschlik (Springer Verlag) discussed with us the possibility to publish a series about Fungi. We both were at first somewhat reserved, but after a comprehensive discussion this idea looked promising. In analogy to another series by Springer Verlag entitled *The Prokaryota* we decided to name the new series *The Mycota*.

Then Paul Lemke and I created a program involving seven volumes covering a wide area of Mycology. The first volume was presented in 1994 at the Fifth International Mycological Congress in Vancover (Canada). The other volumes followed step by step. After the early death of Paul Lemke (1995) I proceeded alone as Series Editor. Since evidently the series was well accepted by the scientific community and since the broad area of Fungi was not completely covered, it was decided to proceed with eight more volumes. In addition, in the following years second editions of eight volumes were published.

Now we present *Volume XV*. This will be the last volume of this series. As its title "*Physiology and Genetics: Selected Basic and Applied Aspects*" expresses, it contains special papers of various fields of Mycology which have been missing in the previous volumes.

Now, after 20 years of editing this series and at the age of 85 years, I guess it is the right time to terminate my editorship. I would like to express my sincerest thanks to all the volume editors, to the numerous authors for their successful cooperation and last but not least to Joan Bennett who supported me in editing three volumes.

I would also like to thank Springer Verlag, represented by Drs. Czeschlick and Schlitzberger for their support and cooperation.

I hope that *The Mycota* will also in the future find the interest of mycologists and other scholars interrested in Fungi and maybe some more second editions.

Bochum, Germany — Karl Esser
May 2009

Volume Preface

More than 120 000 different fungal species have been described and it is estimated that there exist more than 1.5×10^6 species. Fungi have adopted many different ways of living in very diverse habitats as saprophytes, pathogens, symbionts or endophytes. Fungi and their products are used for the fermentation and processing of food and feeds, for biological control and for the production of vitamins and amino acids. Some of their secondary metabolites are used in medicine, e.g. as antibiotics, immunosupressants, cholesterol-lowering drugs or as agrochemical fungicides. Recently, progress in the field of mycology has been substantial due to new methodological approaches and technologies, many of them DNA-based, strongly adding to the motivation to compile a new volume of "*The Mycota*".

Mycota XV "*Physiology and Genetics: Selected Basic and Applied Aspects*" provides a selection of state of the art reviews in traditional and new fields of mycology. As addressed in Chap. 1, DNA sequencing of functionally conserved homologue genes has resulted in new approaches to taxonomy, which hopefully will result in phylogenetic trees and taxonomic groups which can be understood and accepted by the majority of mycologists. As a homothallic fungus *Sordaria macrospora*, producing only sexual meiospores and no asexual conidia, is an ideal model for studying all stages of fruiting body formation and development. The application of several genetic tools allows advanced studies of genetic interactions controlling developmental plasticity (Chap. 2). Chapter 3 summarizes the current knowledge of *inteins*, internal protein sequences, which are "selfish elements" in fungal genomes. They are transcribed and translated together with their host protein and excised at the protein level. Chapter 4 gives an overview of fungal apoptosis in yeasts and filamentous fungi. Similarities and differences between fungal and mammalian apoptosis are discussed and the role of apoptosis in development and ageing are described and evaluated. A broad view on ways and means of the vegetative and sexual interaction of fungal colonies as well as the communication of fungi with bacteria, plants and animals is offered in Chap. 5. In the interaction of yeasts killer toxins play an important role. Their structures, modes of action and resistance as well as possible applications are discussed in Chap. 6. Chapter 7 deals with aspects of evolutionary and ecological interactions of fungi and insects; and Chap. 8 offers an insight into the occurrence and metabolites of endophytic fungi. Not all compounds isolated from plants are genuine plant metabolites but are produced by fungi. Thus the ergoline alkaloids present in *Convolvulaceae* are produced by fungi living in close association with secretory glands on the leaf surface (Chap. 9). Basidiomycetes are a rich source of unique secondary metabolites in most cases not found in other fungi. Chapter 10 offers a survey of new compounds isolated within the past decade, with special emphasis on bioactive metabolites. Genome-wide approaches to identify genes or gene products essential for the establishment of pathogenic interactions between plant host and

fungal pathogen are discussed in Chap. 11. In addition, the authors stress the important role of small molecules in identifying and validating new targets for fungicides. Helminths can pose serious problems to animal and human health. It is therefore quite remarkable that fungi produce low molecular weight compounds specifically interfering with reactions not present in the mammalian hosts, paving the way to non-toxic medications or agrochemicals (Chap. 12). Chapter 13 describes the occurrence, structures and biological activities of peptides and depsipeptides produced by fungi and discusses the importance of these compounds as lead compounds for agricultural and pharmaceutical applications. The sequencing of whole fungal genomes has revealed that there are many more genes supposedly coding for secondary metabolites than there are compounds already isolated and characterized. As can be seen in Chap. 14, homologue overexpression of a regulatory gene construct can indeed lead to silent gene expression and production of the corresponding metabolite. Chapters 15–17 deal with genes, enzymes and products of important biogenetic pathways. Nonribosomal peptide synthetases of fungi differ in some major aspects from the corresponding bacterial enzymes. The same is true for fungal polyketides which constitute a large part of the fungal secondary metabolome. The importance of the detrimental mycotoxin ochratoxin A for human health and the alimentary industry was recognized only recently. The investigation of its biosynthesis and regulation is important for developing strategies for its avoidance in food and feed. Chapter 18 is devoted to genetic and metabolic engineering in fungi, key areas of research aiming at the improved application of these organisms in biotechnology.

We do hope that readers enjoy reading this volume of *The Mycota*. We are very grateful to the contributing authors, whose expertise and efforts have made this project possible. We thank Dr. Andrea Schlitzberger of Springer Verlag for her support and engagement during the preparation of this volume.

Kaiserslautern and St. Ingbert, Germany
May 2009

Timm Anke
Daniela Weber
Volume Editors

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