Handbook of Applied Mycology

Foods and Feeds

Volume 3

edited by
Dilip K. Arora
K.G. Mukerji
Elmer H. Marth

Handbook of Applied Mycology

Volume 3: Foods and Feeds

edited by

Dilip K. Arora
Banaras Hindu University
Varanasi, India

K. G. Mukerji
Department of Botany
University of Delhi
Delhi, India

Elmer H. Marth
University of Wisconsin—Madison
Madison, Wisconsin

MARCEL DEKKER, INC. New York • Basel • Hong Kong

ISBN 0-8247-8491-X

This book is printed on acid-free paper.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Current printing (last digit):
10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

Handbook of Applied Mycology
Volume 3: Foods and Feeds

Handbook of Applied Mycology

Series Editor
Dilip K. Arora

Banaras Hindu University
Varanasi, India

Volume 1	Soil and Plants, edited by *Dilip K. Arora, Bharat Rai, K. G. Mukerji*, and *Guy R. Knudsen*
Volume 2	Humans, Animals, and Insects, edited by *Dilip K. Arora, Libero Ajello*, and *K. G. Mukerji*
Volume 3	Foods and Feeds, edited by *Dilip K. Arora, K. G. Mukerji*, and *Elmer H. Marth*
Volume 4	Fungal Biotechnology, edited by *Dilip K. Arora, Richard P. Elander, and K. G. Mukerji*
Volume 5	Mycotoxins in Ecological Systems, edited by *Deepak Bhatnagar, Eivind B. Lillehoj, and Dilip K. Arora*

HANDBOOK INTRODUCTION

Applied mycology is one of the most stimulating and rapidly evolving areas of the biological sciences. It encompasses many facets of agricultural, industrial, pharmacological, medical, and food sciences, all of which include organisms or processes that are subject to biotechnological manipulation. However, published information on the practical significance of fungi is fragmented in many specialized publications. There is an obvious need for a comprehensive treatment of the basic principles, methods, and applications of mycology as an applied, integrated, and multidisciplinary subject. The potential usefulness of such a handbook is suggested by the practical significance of mycological research, by the uniqueness and increasing coherence of the field's conceptual directions, and by the international scope of the subject. These five volumes of the *Handbook of Applied Mycology* attempt to fulfill this need. These volumes present and collate the major aspects of applied mycology and are designed as a modern standard reference for the work in this field.

In developing these volumes we decided that the treatment of the subject should be comprehensive but concise enough to enable completion within a set of five volumes published closely together rather than a continuous encyclopedic series taking several years to complete. However, this does not preclude the possibility of publishing supplementary volumes, as appropriate, to communicate new information. Further, we wanted to develop a treatise that would offer a detailed overview of applied aspects of mycology in terms of its theoretical, methodological, and empirical contributions. By emphasizing the interdisciplinary aspects of the subject, we hope that the handbook will highlight recent prospective linkages among diverse research paradigms.

Since the information presented in these volumes is expected to serve students as well as veterans in the field, both basic and advanced specialized materials are included. In addition to addressing researchers in mycology, the handbook will prove useful to a broad spectrum of professional groups, including biotechnologists, medical scientists, molecular biologists, food scientists, pharmacologists, soil microbiologists, microbial ecologists, and plant pathologists. Each chapter is intended to provide a balanced view of the current state of knowledge within an expanding field of interest. The selection of the chapters and overall organization of the book

were guided by several insightful colleagues from around the world. These volumes were authored by a large team of international experts. Every chapter within the five volumes has been edited by a distinguished group of scientists. As a result, nine editors and over 200 authors representing 20 countries have contributed to this project.

Because of the multidisciplinary nature of the subject, judgment had to be made about the relative importance and significance of the hundreds of potential topics related to applied mycology. Also, a balance had to be struck between comprehensive coverage and intellectual appeal of the field. Readers should find this work useful for in-depth information and as a route to additional information contained in its critical discussions and wide range of references provided. Each of the five volumes is intended to be self-contained. Therefore, some degree of duplication of materials, especially basic principles, is inevitable.

No work of this magnitude can be accomplished without the support and contributions of many individuals. The planning and eventual production of this handbook were a collaborative effort. I am deeply indebted to the group of coeditors and many colleagues who have assisted me throughout all stages of the project. I also appreciate the dedication and hard work of the chapter authors, who cooperated persistently in the production of these volumes. I acknowledge with gratitude the support of M. V. Wiese, N. W. Schaad, S. Stotzsky, J. S. Singh, Bharat Rai, R. S. Upadhyay, V. S. Jaiswal, B. Thompson, Jon Olson, A. K. Pandey, Rejeev Gaur, and Shusma Gupta, whose interest and cooperation have been invaluable to me. I am grateful to the University of Idaho for providing assistance in the form of staff support and facilities, and the Banaras Hindu University, India, for granting leave during the period I edited these volumes. I appreciate the encouraging support provided by Ms. Sandra Beberman of Marcel Dekker, Inc., at all stages of production of this book. My gratitude is expressed to my teacher, J. L. Lockwood, and to my parents, whose example has always been my guiding principle. Finally, to Meenakshi, Sidhartha, and Gautam for their patience, encouragement, and willingness to forgo other interests so that the handbook might be completed, I am grateful.

Dilip K. Arora

PREFACE

Molds probably spoil more food than any other group of microorganisms. Some molds produce highly toxic mycotoxins, whereas other molds are used in fermentations to produce a variety of foods. Yeasts are also important as spoilage organisms and in fermentations, particularly for the production of alcohol.

In spite of the importance of these fungi, they have traditionally received only minimal attention in textbooks on food microbiology and hence by food microbiologists. This began to change when some molds were recognized as able to produce highly toxic secondary metabolites during growth on foods and feeds. Afterward, scientists having diverse backgrounds, including food microbiologists, mycologists, physiologists, toxicologists, and others, became interested in and studied molds. Thus, much new information about this group of microorganisms became available. Other investigators, for different reasons, studied yeasts and developed new information.

Such new information, when blended with older knowledge, is the substance of a book. Indeed, several books on food mycology appeared during the 1980s. However, the subject of fungi as related to foods is so broad that no single book on this subject can provide all the available information. Consequently, this volume supplements the other books on this subject by providing much information that is not readily available elsewhere. The book opens with a taxonomy of fungi in foods and feeds and then considers ecology, spoilage, and mycotoxin production by fungi in foods and feeds. This is followed by a series of discussions on xerophilic fungi and on fungi as they affect grain, vegetables, fruits, dairy products, nonproteinaceous and proteinaceous fermented foods, and food processing. Edible fungi including mushrooms and single-cell proteins are discussed as are products and uses of yeasts. Control of fungi through acceptable food additives is also considered.

We hope that this book will be useful to food and grain scientists, food microbiologists, mycologists with an interest in foods, persons in regulatory agencies who must deal with both the problems and benefits resulting from molds in foods and feeds, and students who wish to gain an appreciation for the importance of fungi in foods and feeds.

Our thanks go to the contributors for their efforts in providing thorough and up-to-date discussions of the various topics. We also thank Ms. Sandra Beberman and Mr. Andrew Berin of Marcel Dekker, Inc., for their efforts in turning manuscript pages into printed pages.

Dilip K. Arora
K. G. Mukerji
Elmer H. Marth

CONTRIBUTORS

Dilip K. Arora Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi, India

Zakia Bano Fruit and Vegetable Technology Department, Central Food Technological Research Institute, Mysore, India

Ramunas Bigelis Biotechnology Division, Amoco Research Center, Amoco Technology Company, Naperville, Illinois

Robert E. Brackett Department of Food Science and Technology, Georgia Agricultural Experimental Station, The University of Georgia, Griffin, Georgia

Marlene A. Bulgarelli* Department of Food Science and Technology, Georgia Agricultural Experimental Station, The University of Georgia, Griffin, Georgia

Shu-Ting Chang Department of Biology, The Chinese University of Hong Kong, Shatin, Hong Kong

Clyde M. Christensen Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota

Jens C. Frisvad Department of Biotechnology, The Technical University of Denmark, Lyngby, Denmark

Alison Hamer Plant Pathology Department, Agricultural and Food Research Council Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Hertfordshire, England

---

**Present affiliation:* Center for Infectious Diseases, Division of Bacterial Diseases, Meningitis and Special Pathogens Branch, Centers for Disease Control, Atlanta, Georgia

Ailsa D. Hocking Food Research Laboratory, Division of Food Processing, Commonwealth Scientific and Industrial Research Organization, North Ryde, New South Wales, Australia

Surinder Singh Kahlon Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India

John Lacey Plant Pathology Department, Agricultural and Food Research Council Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Hertfordshire, England

Michael B. Liewen Microbiology and Regulatory Services, General Mills, Inc., Minneapolis, Minnesota

Naresh Magan Biotechnology Centre, Cranfield Institute of Technology, Cranfield, Bedford, England

Ian C. Marfleet* Biotechnology Centre, Cranfield Institute of Technology, Cranfield, Bedford, England

Elmer H. Marth Department of Food Science, University of Wisconsin—Madison, Madison, Wisconsin

Tilak W. Nagodawithana Technical Center, Universal Foods Corporation, Milwaukee, Wisconsin

S. Rajarathnam Fruit and Vegetable Technology Department, Central Food Technological Research Institute, Mysore, India

Nannapaneni Ramakrishna Plant Pathology Department, Agricultural and Food Research Council Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Hertfordshire, England

Robert A. Samson Centraalbureau voor Schimmelcultures, Baarn, The Netherlands

Don F. Splittstoesser Department of Food Science and Technology, New York State Agricultural Experimental Station, Cornell University, Geneva, New York

Tamotsu Yokotsuka Research Division, Kikkoman Corporation, Noda City, Chiba, Japan

Ahmed E. Yousef† Department of Food Science, University of Wisconsin—Madison, Madison, Wisconsin

---

Present affiliations:

*School of Applied Biology, Lancashire Polytechnic, Preston, England

†Department of Food Science, Ohio State University, Columbus, Ohio

Handbook of Applied Mycology

Volume 3: Foods and Feeds

CONTENTS

1

TAXONOMY OF FILAMENTOUS FUNGI IN FOODS AND FEEDS

ROBERT A. SAMSON *Centraalbureau voor Schimmelcultures, Baarn, The Netherlands*

JENS C. FRISVAD *The Technical University of Denmark, Lyngby, Denmark*

DILIP K. ARORA *Banaras Hindu University, Varanasi, India*

I. INTRODUCTION

The importance of the filamentous fungi in food microbiology has gained general recognition. The importance of mutual understanding of food mycology and taxonomy as they relate to food processing and preservation has been stressed by Beuchat [1] for the quality control of foods and feed. The identification of the contaminating mycoflora is essential. The determination up to species level will provide important data about the biology and biochemistry of the organism, including the possible production of mycotoxins. Books and manuals for identification and isolation of foodborne fungi are available [2-4]. However, in the past the identification of foodborne fungi has often been neglected possibly due to lack of appropriate literature and techniques for carrying out a reliable identification, or the researcher, usually a microbiologist trained as a bacteriologist, lacked mycological training. Consequently, fungal isolates were not thoroughly identified and were incorrectly named. The literature on foodborne fungi has greatly suffered from the misidentified isolates. In some instances this has led to serious errors, e.g., isolates which were incorrectly identified as toxinogenic species. The taxonomic schemes of the most important foodborne genera such as *Penicillium*, *Aspergillus*, and *Fusarium* were varied and confusing. For many decades different species concepts were used. Although the research on the taxonomies of these genera is improving, mostly with the aid of modern techniques, the systematics of these genera are still in a state of flux. Recent international collaboration on the systematics of *Penicillium* and *Aspergillus* has improved the standardization of the species concepts, and in the important groups a consensus has been reached [5,6].

Presently the taxonomy of most foodborne genera is elucidated, and hence, identification up to species level is possible. Samson [7] has

compiled the genera that occur in foods and feeds. There is a small difference between the two lists compiled by Samson et al. [8] and Pitt and Hocking [4] with respect to certain genera, and this may be explained by deviation in geographical distribution (Australia vs. Europe) and the different concept of food mycology.

In this chapter the identification of the filamentous fungi occurring in foods and feeds is discussed in brief. References to conventional methods of identification are given, while new approaches are shortly mentioned. A list of common and less common genera with the most relevant literature for identification is provided.

II. OCCURRENCE

Although there are many papers describing the mycoflora of specific products and commodities, only a few general lists of foodborne fungi and their specific substrates exist. Williams [9] lists spoiled foods from which species of *Penicillium* and *Aspergillus* have been isolated. Frisvad [10] has compiled a similar list, and the ecology of foodborne fungi is discussed in detail by Pitt and Hocking [4] and Frisvad and Samson (see Chapter 2).

During the last 15 years, *Penicillium* and *Aspergillus* have dominated the spoilage mycoflora of moldy food samples examined in our laboratory. Williams and Bialkowska [11] reported that from 294 samples with visible growth of one or more mold species, *Penicillium* accounted for 53.1% of isolates, *Aspergillus* and associated teleomorphs 15.1%, *Cladosporium* 8.9%, *Mucor* 8.5%, *Rhizopus* 4.8%, and other genera 9.6%. Of the Penicillia, subgenus *Penicillium* (90.1%) was dominant.

It is hoped that other laboratories will be encouraged to publish ecological data on foodborne fungi, so that further information may become available on the relationship between species and pattern of spoilage. This would not only supplement information already available about foods at risk from particular mold species, but would also highlight those ubiquitous species whose presence gives no information on likely contamination source.

III. IDENTIFICATION

A. Morphology

For the classification of filamentous fungi, morphology is still used as a primary criterion. This often facilitates a rapid identification, when the sporulating structures of the molds are present in or on the food product. Examination of the morphological structures can be easily carried out by making a simple microscope preparation and studying it with a well-equipped light microscope. However, traditional morphology-based taxonomy is now supplemented by other biochemical and physiological characteristics, in order to produce more sensitive taxonomies appropriate to the demands now being made by those who use many of the fungi concerned.

Until recently most mycologists were not interested in using data obtained by morphological, physiological, and biochemical studies of foodborne fungi for the purpose of numerical taxonomy. Numerical taxonomy

can be a useful tool for grouping of fungi that have large number of characters in common. This technique encompasses a polythetic approach and can provide much information and indicate strain variations.

For identification, several general textbooks describing the most important fungal genera and species found in foods should be consulted [12-20].

Most foodborne Ascomycotina belong to the order Eurotiales (= Plectomycetes) and are characterized having an anamorph. These anamorphs belong to *Aspergillus, Penicillium, Basipetospora,* and other hyphomycetes. Benny and Kimbrough [21] and von Arx [12] have provided keys to the genera of the Eurotiales.

The Deuteromycotina includes the most important genera found in foods. The taxonomy of genera of these fungi is based on characters of conidiogenesis. Several reviews and classifications based on conidial development are available [22-24]. For the identification of the most common foodborne taxa, the reader is referred to Pitt and Hocking [4] or Samson and van Reenen-Hoekstra [3].

Coelomycete species, which are mostly accommodated in the Deuteromycotina, are usually not true saprobic contaminants, but often occur as weak plant pathogens [12,25]. In addition, many members of the Coelomycetes may not grow on selective laboratory media and will not sporulate adequately on agar media.

The classifications of foodborne yeasts will not be treated here. Published information about the given modern classification of yeasts can be found elsewhere [26-28].

B. Cultivation

For the morphological examination it is important to culture each species on an appropriate medium to achieve typical growth and sporulation. Malt-extract agar (MEA) and/or oatmeal agar (OA) are suitable media for most species. A number of species require special media such as potato-carrot agar (PCA), Czapek yeast autolysate agar (CYA), carnation leaf agar (CLA), etc. Recommendations and instructions for cultivation and media are given by Samson and van Reenen-Hoekstra [3] (Table 1). For other mycological media see King et al. [29], Gams et al. [30], and Stevens [31].

Most fungi can be incubated in light or in the dark at 25°C and identified after 5-10 days. For instance, *Fusarium, Trichoderma,* and *Epicoccum* show typical sporulation in diffuse daylight. Fungi such as *Phoma* should be cultivated in darkness followed by a period of alternating darkness/diffuse (day)light. To stimulate sporulation, irradiation with near UV ("black" light: greatest effect at 310 nm with a maximum emission about 360 nm) can be useful. The details of techniques for examining foodborne fungi are given by Samson and van Reenen-Hoekstra [3].

C. Secondary Metabolites

Patterns of secondary metabolites have now become an effective taxonomic tool, especially when used in conjunction with traditional taxonomy. Recently, Filtenborg, Frisvad, and coworkers have developed and described a very simple screening method for toxigenic fungi [32-37].