BIOTECHNOLOGY HANDBOOKS • 7

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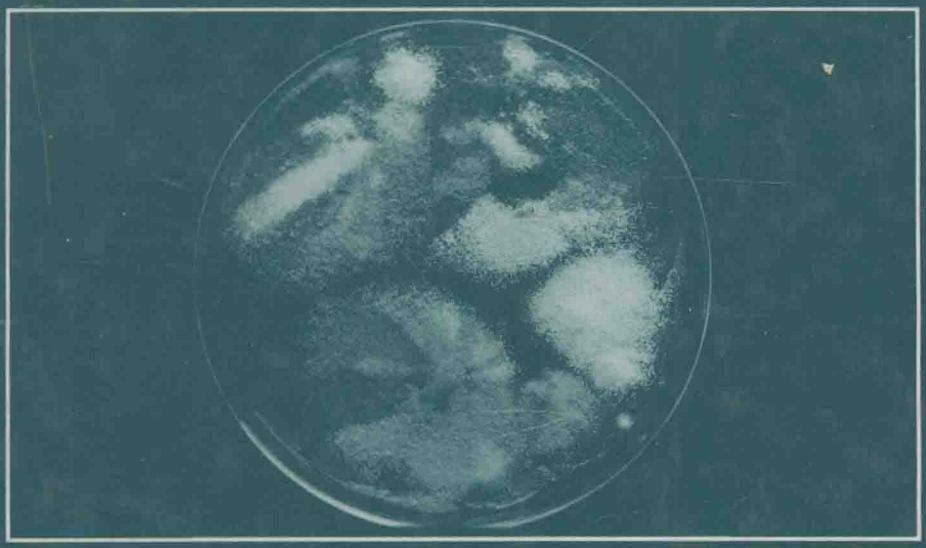

# *Aspergillus*

Edited by J. E. Smith

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*Glasgow, Scotland*

Plenum Press • New York and London

Library of Congress Cataloging-in-Publication Data

Aspergillus / edited by J.E. Smith.
p. cm. -- (Biotechnology handbooks ; v. 7)
Includes bibliographical references and index.
ISBN 0-306-44545-X
1. Aspergillus--Biotechnology. 2. Fungi--Biotechnology.
I. Smith, John E. II. Series.
TP248.27.F86A873 1993
660'.62--dc20 93-39484
CIP

ISBN 0-306-44545-X


A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013



Printed in the United States of America

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*PHLS Centre for Applied Microbiology and Research*
*Division of Biotechnology*
*Salisbury, Wiltshire, England*

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# Preface

The genus *Aspergillus* has a worldwide distribution and is one of the most common of all groups of fungi. They are possibly the greatest contaminants of natural and man-made organic products, and a few species can cause infections in man and animals. The aspergilli are also one of the most important mycotoxin-producing groups of fungi when growing as contaminants of cereals, oil seeds, and other foods. Not all aspergilli are viewed as troublesome contaminants, however, as several species have had their metabolic capabilities harnessed for commercial use.

The aspergilli have long been associated in the Far East with the koji stage of several food fermentations, particularly soy sauce and miso, and subsequently as a source of useful enzymes. The ability of these fungi to produce several organic acids, especially citric acid, has created major industrial complexes worldwide. Traditional methods of strain development have been extensively studied with the industrial strains, while more recently, recombinant DNA technology has been applied to the aspergilli with emphasis on heterologous protein production.

In compiling this book, I have been fortunate to have the full enthusiastic involvement of the authors, and to them I extend my very grateful thanks for mostly being on time and for producing such readable and authoritative chapters.

Collectively, we hope that our efforts will strengthen the scientific understanding of this intriguing group of filamentous fungi and further their use in the field of biotechnology.

John Smith

*Glasgow, Scotland*

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Chapter 6

# Taxonomy—Current Concepts of *Aspergillus* Systematics

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ROBERT A. SAMSON

## 1. INTRODUCTION

Species of *Aspergillus* belong to the first fungal organisms that were cultivated on artifical media and studied for their biochemical properties, and they are one of the most common fungi in man's environment. Since ancient times, *Aspergillus* species have been used in fermentation of food in Japan and other Asian countries, and the early discovery of their ability to produce organic acids was made at the turn of the century. It is therefore not surprising that the aspergilli used or encountered in biotechnology play a significant role.

The first description of *Aspergillus* dates from 1727, when P. A. Micheli provided a meager diagnosis and simple illustrations, but a clear depiction of the characteristics of the genus. After this first description, many taxa were described, and Thom and Church (1926) were the first to revise the genus *Aspergillus* with the acceptance of 69 species divided into 11 groups based on colony morphology and characteristics of the aspergillum. Later, Thom and Raper (1945) comprised 77 species with 10 varieties placed in 14 groups. In their monographic treatment, Raper and Fennell (1965) accepted 132 species subdivided into 18 groups. The generic and species concept was weli circumscribed, and this monograph is still accepted. Since 1965, many new taxa have been described, and the validity of these species has been reviewed by Samson (1979; 1992). The genus now contains more than 200 species.

Two International Workshops on *Penicillium* and *Aspergillus* (Samson and Pitt, 1985, 1990) had a great impact on the current taxonomic

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*Aspergillus*, edited by J. E. Smith. Plenum Press, New York, 1994.

schemes of *Aspergillus*. The contributions have also revealed the significance for the systematics of this important genus of a multidisciplinary approach including biochemistry, molecular biology, and serology. Recently, several molecular studies on problematic but important aspergilli have been performed, and the potential taxonomic value is apparent. An accurate, fast detection of species and isolates might be possible in the near future. This will be particularly required for *Aspergillus* strains that have undergone various modifications for specific biotechnogical applications. Often, these strains will not produce typical morphological structures any more, and recognition will be possible only at the molecular level. At this time, however, the results of molecular studies need careful evaluation with a good comparison of morphological characters.

## 2. CRITERIA FOR CLASSIFICATION AND IDENTIFICATION

### 2.1. Morphology

Morphological structures are still important characters for classification. *Aspergillus* species typically produce the aspergillum, a conidiophore with an aseptate stipe terminating in a vesicle, on which the conidiogenous cells (phialides and metulae) are borne. These phialides produce conidia in long, dry chains with different pigmentation and ornamentation. These structures can be readily seen with a light microscope equipped with an oil immersion lens. In addition to the typical structure of the aspergillum, sclerotia and fruiting bodies with Hülle cells that can be typical for the species can also be produced. Also used for species delimitation, however, are colony color and diameter, characteristics of the vegetative mycelium, exudate (droplets on colony surface), reverse, and soluble pigment (colors in the agar around the colony).

Scanning electron microscopy (SEM) has been applied in the systematics of *Aspergillus;* in particular, the ornamentation of thick-walled ascospores allows examination by SEM in the unfixed, air-dried state. Conidia and other structures are less resistant to shrinkage, and artifacts may develop during fixation and examination of conidia by SEM.

Most aspergilli can be readily identified when cultivated on Czapek and malt extract agars. Samson and Pitt (1985) recommended Czapek yeast agar (CYA) and malt extract agar (MEA) for most anamorphic species. For teleomorphic species, cornmeal agar, oatmeal agar, and CBS malt agar were recommended. For xerophilic species, Czapek agar or CYA with 20% sucrose or malt yeast extract 40% sucrose agar should be used. Cultures should be incubated for 5–7 days at 25°C for anamorphic species and 10–14 days for teleomorphic species.

Some specialized media are used for the isolation and identification of potentially aflatoxigenic aspergilli. Bothast and Fennell (1974) and Pitt *et al.* (1983) proposed Aspergillus Differential medium and Aspergillus Flavus and Parasiticus Agar, respectively, which make detection of *A. flavus* and *A. parasiticus* possible within 3 days. Another medium containing the antibiotic bleomycin distinguishes *A. parasiticus* from *A. sojae* (Klich and Mullaney, 1989). On this medium, growth of both species is somewhat reduced, but *A. sojae* produced very restricted colonies, while *A. parasiticus* colonies were at least 3 mm in diameter within 6 days. These selective media are especially appropriate for fast detection of mycotoxinogenic isolates in food commodities.

Practical laboratory guides for identification of common aspergilli that are based primarily on morphology and colony characters were published by Pitt and Hocking (1985), Samson and Van Reenen-Hoekstra (1988), Klich and Pitt (1988a), and Tzean *et al.* (1990).

## 2.2. Other Methods for Classification and Identification

Besides the convential characters of the colony and morphological structure, several new approaches have been investigated (Samson and Frisvad, 1991a,b; Samson, 1992).

The use of enzyme profiles has been studied for a limited number of *Aspergillus* isolates by a number of investigators (Nealson and Garber, 1967; Nasuno, 1971, 1972a,b, 1974; Kurzeja and Garber, 1973; Cruickshank and Pitt, 1990; Sugiyama and Yamatoya, 1990; Yamatoya *et al.*, 1990). In some cases, taxonomic relationships could be elucidated, but as yet this technique has not provided a reliable system for identification. However, profiles of secondary metabolites including mycotoxins have been shown to be useful in *Aspergillus* systematics. Samson *et al.* (1990) clearly distinguished nine taxa within the genus *Neosartorya* by ascospore morphology and the profile of secondary metabolites. Frisvad and Samson (1990) compared the morphological characteristics of many isolates of *A. fumigatus* and related taxa with their secondary metabolite profiles and were able to confirm the existing species delimitation. Klich and Pitt (1988b) investigated the relationship between species and secondary metabolite production in Section *Flavi* and found a good correlation between the production of aflatoxins $B_1$ + $B_2$, $G_1$ + $G_2$, and cyclopiazonic acid and the morphological characteristics of the species.

The distribution of ubiquinone systems, often in combination with electrophoretic comparison of enzymes, has been used by several Japanese researchers (Kuraishi *et al.*, 1990; Matsuda *et al.*, 1992). These chemotaxonomic approaches indicate relationships between groups, but