moulds and filamentous fungi in technical microbiology

OLGA FASSATIOVÁ

progress in industrial microbiology

volume 22

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Department of Cryptogamic Botany
Charles University, Prague

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PREFACE

The "filamentous fungi" may be defined as microscopic eukaryotic organisms producing fine filamentous growth on various substrates. They belong to fungi (*Mycota*) and include pin-moulds (order *Mucorales*), among which *Mucor* is the most common genus, and the order *Moniliales* (*Hyphomycetes*) of the imperfect fungi (*Deuteromycetes*), with genus *Penicillium* as the most common genus representative. In addition, *Mycelia sterilia* (nonfertile mycelia without any spores and belonging to the imperfect fungi) are included here. The mould-like character, i.e. fine, filamentous growth, may also be typical of the mycelium of *Basidiomycetes*. However, the latter are outside the scope of this volume.

The degradative activity of the filamentous fungi and the products of their metabolism are utilized in many and varied research and industrial areas. Species identification and knowledge of life cycles are therefore required for further research, and for the use of these organisms. For scientific work a number of taxonomic monographs and handbooks on phytopathology, soil mycology and medical mycology are available. However, a handbook of saprophytic microscopic fungi that contaminate food and agricultural and industrial products that would provide more detailed information for microbiologists working in the food industry, public health, human and veterinary medicine and in agricultural research is still missing, in both the Czechoslovak and the foreign literature. On the basis of many years of experience I decided to write a handbook that would include modern approaches to the classification of pin-moulds and *Hyphomycetes* and thus facilitate the identification of common saprophytic species.

A special part of the text includes descriptions of 31 species of the order *Mucorales*, 126 species of the order *Moniliales* and 2 species of *Mycelia sterilia*, which can be identified according to genus- and species-organized keys. Of the several thousand species, only the saprophytic species occurring as contaminants in the food industry, agriculture and medicine are included. In all these areas saprophytic microorganisms are considered, from the point of view both of deterioration of products and of their hygienic and health hazards. Therefore, descriptions are also upplemented by remarks on ecology and biochemical properties.

As already mentioned, the species were chosen according to their practical importance. Thus, the keys for their identification are selective, and the reader should be aware of the fact that errors may occur when identifying a species that has not been included. However, due to technical reasons and difficulties in identification, it was not possible to include all the species that have been described so far. The monographs cited should help when trying to identify a species which is not included in this handbook. As it was intended as a handbook for identification rather than a taxonomical monograph, synonyms are given only for selected species, particularly those that are most commonly used. A detailed list of recommended literature is always given, in *Hyphomycetes* (*Moniliales*) after each genus, in pin-moulds (*Mucorales*) after the whole order. Descriptions of almost every species are supplemented with line drawings, and some species are also illustrated by micro- and macrophotographs. The methodological part includes laboratory techniques of isolation, cultivation and identification of these microscopic fungi.

I wish to thank Dr. Zora Boušková for preparing line drawings according to models in the literature, and for drawing the plates.

OLGA FASSATIOVÁ

CONTENTS

I INDUSTRIAL AND ECONOMIC SIGNIFICANCE OF MICROSCOPIC FUNGI

In the economy of nature, moulds and fungi are involved in the production of humus and many of them live in the soil in contact with roots of higher plants. Therefore, the soil is a permanent reservoir of filamentous organisms. Saprophytic species play an important role in the processes of mineralization. However, the soil also contains parasites and pathogens which survive in the form of permanent spores or in the saprophytic phase of their life cycle. These species infect viable plant or animal organisms and, hence, function as a regulatory agent of some populations in the biocenosis. When cultivated plants are invaded by them, these fungi become an unfavourable factor in forestry and agriculture. Saprophytic species may also attack stored products or fresh or preserved foods and cause significant damage. By degrading sugars, proteins and lipids the quality of these products may be altered. Some of them reduce the content of vitamins. It is known that flour invaded by some filamentous fungi is so much changed by their proteolytic enzymes that it cannot be used in bakeries. These organisms also deteriorate the life environment of man and by their metabolites (toxins in particular) or other activities they can impair the state of health of humans and animals. The significant adaptability of moulds and filamentous fungi to nutrients, physical and climatic conditions, as well as their simple propagation, allows them to colonize new substrates and, thus, invade whole areas of our environment.

On the other hand, filamentous fungi may also play a positive role in various industrial processes. Above all, some areas of the food industry cannot exist without them. They are used in bread production, in dairies and in the production of alcoholic beverages, all of which have a long tradition in the history of mankind. Production of cheese by the proteolytic activity of some penicillia should also be mentioned here. Microscopic fungi are also used in the meat industry in the production of durable sausages.

The chemical industry utilizes the filamentous fungi for their ability to synthesize organic acids, vitamins, hormones and various growth-supporting compounds. *Aspergillus niger*, one of the most common species of *Hyphomycetes*, is used in the production of citric acid. Some species of the genus *Fusarium* synthesize stimulators, termed gibberellins, that are used to support growth of some cultivated plants, but also in the brewing industry in the germination of barley.

The pharmaceutical industry began the production of antibiotics by the discovery of penicillin produced by *Penicillium chrysogenum* and *Penicillium notatum*. Antibiotics are also produced by a number of other penicillia but many are cytotoxic. A series of species of filamentous fungi can transform steroid hormones by enzyme oxidation-reduction and hydrolysis. This property is utilized in the synthesis of pharmacological steroid compounds and their derivatives.

The moulds and filamentous fungi are presently considered as agents that can be used in decontaminating polluted waste waters. Water engineers all over the world have to solve problems of polluted waters, and many saprophytic filamentous fungi can degrade compounds flowing with waste waters to the rivers and, thus, contribute to their cleaning.

In the last two decades problems of toxicity of many filamentous fungi attracted much attention. These problems, particularly in agriculture and the food industry, became of utmost importance. In addition to deterioration of agricultural and food products, a possible intoxication must be considered. The study of mycotoxins of moulds and fungi started after 1960, when a massive destruction of young turkeys occurred in England. It was later found that the turkeys were fed peanut flour containing *Aspergillus flavus*. As it was later demonstrated, this filamentous fungus produces aflatoxins that are more toxic than toxins of higher fungi even at lower concentrations and also exhibit carcinogenic effects. At present, tens of species of filamentous fungi are known to produce mycotoxins and are commonly present in stored grain and flour, oily seeds, dairy products, meat, eggs, animal and vegetable fats, fruits, vegetables, beverages etc. Mouldy fodder can also be toxic.

Most toxigenic fungi belong to genera *Aspergillus, Penicillium* and *Fusarium* (*Moniliales*). It is known that these fungi produce toxins only on certain substrates and under specific conditions. In general, the production of mycotoxins is more intensive on food rich in carbohydrates. The mycotoxins penetrate into foodstuffs and various cultural plants without changing their appearance. It is also problematic that these toxins are mostly heat-stable. Therefore, in the protection of foodstuffs and fodder we are practically limited to preventing the infection.

For the survival of filamentous fungi in industrial processes, their temperature optimum is very important. Psychrophilic mycoflora can survive even on food maintained in cold stores (e.g. *Cladosporium, Alternaria, Botrytis*), whereas thermophilic mycoflora survive at temperatures up to 90 °C (*Byssochlamys fulva*). During the sterilization of some food products insufficient heat penetration often allows the survival of many thermophilic species.

In nature the thermophilic species are involved in degradative processes, during the spontaneous combustion of hay, straw, peat, during overheating of compost, fermentation of tobacco and in sugar-beet and corn silage. Mammalian pathogens (the temperature optimum of which is 37—40 °C) include *Aspergillus fumigatus* and *Rhizomucor pusillus*.

Some moulds and filamentous fungi are highly tolerant to extreme values of hydrogen ion concentration in the substrate. They can be found in a very acidic environment, even in concentrated acids, or also in a highly alkaline environment.

We can detect the degradative activity of these microscopic fungi in various situations, when they destroy wood, stored paper, textiles and plastic and various wrapping materials (tropicalization). There are some species which invade resins, gluing together lenses of microscopes and other optical instruments. New construction materials, those produced from crushed plant parts in particular, are highly endangered by the moulds. Many species of the orders *Moniliales* and *Mucorales* utilize oil and oil hydrocarbons as substrates. The filamentous fungus *Cladosporium resinae* which can massively colonize oil reservoirs is an extreme example of adaptation to an unusual substrate.

It follows from the above examples that our environment is really full of filamentous organisms. Therefore, hygienic precautions must be strictly respected. A number of saprophytic or semiparasitic species can cause mycoses and intoxications and quite often allergic diseases. Mainly agricultural workers had previously suffered from the allergic diseases of the respiratory tract. They are quite common at present in Europe and are caused by spores of several species of the genera *Alternaria, Cladosporium, Stemphylium, Penicillium, Aspergillus, Botrytis* etc. These filamentous fungi are present in the open air and also in flats and wet places, where residues of semidegraded organic compounds are present.

It was the aim of this brief introduction to demonstrate the varied activities of fungi, the saprophytic species in particular. The literature references listed below can provide more detailed information.

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II CHARACTERISTICS AND GENERAL TAXONOMY OF FUNGI

Fungi are heterotropic organisms, i.e. they require an organic substrate for their nutrition. They are classified as an independent taxon *Mycota* belonging to *Thallophyta*.

The hypha is the basic unit of the thallus. Only the most primitive moulds have a simple, microscopic vesicular cell (holocarpic cell) capable of all life functions. Hyphae are either with or without septa and form a simple, complex, or highly branched mycelium. Hyphae germinating form spores tend to grow radially on a solid substrate and in the form of pellets in a liquid medium. We recognize the substrate mycelium serving for nutrition and differing in many respects from the aerial mycelium. Haustoria of parasitic fungi are a special example of the substrate mycelium. In higher fungi densely interwoven branched hyphae and their clustering give rise to a special type of tissue (fungal plectenchyme — in fruit bodies of cap-bearing fungi or pseudoparenchyme — in sclerotia, stromata and multilayered walls of the *Ascomycetes*).

Reproductive organs, both sexual and asexual, are formed on the filamentous thallus or in the fruit bodies. The shape and mode of formation of the spores serve as basic taxonomic features. The sexual organs of fungi, called gametangia, are differentiated into male and female types according to their shape (e.g. male antheridium and female oogonium in the *Oomycetes* or male antheridium and female ascogonium in *Ascomycetes*). In other cases gametangia are not differentiated (the so-called isogametangia in *Chytridiomycetes*) or are not produced at all and only fusion of the + and — mycelia takes place (in *Zygomycetes, Basidiomycetes*). The sexual reproduction in fungi thus consists either of the fusion of sexual cells (gametes), whole gametangia or, occasionally, of fusion of plus and minus hyphae. All these processes lead to a zygote, which is diploid, its nucleus or nuclei having a double number of chromosomes. Karyogamy follows either immediately after the plasmogamy in the same cell (most lower fungi and certain *Ascomycetes*) or karyogamy occurs only later — in cells growing out secondarily from the zygote (ascogenic cells or hyphae of *Ascomycetes*). The nuclei of zygotes are subjected to reductive division (meiosis) prior to germination, so that haploidy is restored in the germinating filaments, and hence, also the new thallus. A different type of sexuality occurs in *Basidiomycetes*. The + and — mycelia (the so-called primary mycelia) fuse, giving rise to a dikaryotic mycelium (secondary), from which the fruiting body outgrows. Cells called basidia are formed in the fertile