

Fungi as a Cause of Allergic Disease

SUZANNE GRAVESEN

Diagnoselaboratoriet, Copenhagen S, Denmark

The role of microfungi in the manufacture of bread, beer, and wine has been known and appreciated for centuries.

Less well known is perhaps the industrial value of many common moulds used in the production of antibiotics, enzymes and steroids.

The increase in infections from fungi due to the extensive use of antibiotics and immunosuppressive agents is a problem under current discussion. Attention is also being focused on the very potent nephro- and hepato-toxic metabolites produced in common species such as *Aspergillus* and *Penicillium* which exude these mycotoxins into various animal foodstuffs for example, which if stored under moist conditions become contaminated with moulds. Due to the ubiquitous presence of fungi in nature and the escalating industrial exploitation of common moulds, attention must be paid to occupational allergic problems as well as to allergen sources related to fungi, outside and especially inside the home.

General biological and ecological aspects

Fungi consist of a heterogeneous group of non-photosynthetic plant bodies: unicellular, colonial or filamentous. The cell wall contains the structural macromolecules of chitin and cellulose together with other polysaccharides and specific amounts of proteins and lipids. The polysaccharides make up about 80-90% of the dry weight of the fungal cell. (28, 33).

Nutrition. In contrast to bacteria and algae, fungi are entirely heterotrophic. Their food

demands range over a broad spectrum of organic material: from living plants or animals (the parasitic fungi) to dead organisms and such material as cellulose, paint, or stored products. Due to their various specific and potent enzymatic abilities, the fungi play an important role in nature in the biological recycling process, by degrading organic material to chemically simple components. Table 1 shows some of the enzymes generated from common soil fungi (9).

Humidity. Fungi are able to grow at a relative humidity (RH) below that of bacteria and algae. The competitive ability of fungi is furthered by its ability to respond by sporulating when the RH is lowered. The minimum RH permitting growth varies between 75% and 95% for different species of moulds. Xerophilic fungi such as *Aspergillus penicillioideus* and *Eurotium repens* and a few others are, however, able to grow below 75% RH due to their ability to bind atmospheric water. Xerophilic fungi are abundantly represented in house dust, where they facilitate house dust mites' ingestion of human skin-scales (17).

Temperature. The fungi dealt with in this context are mainly mesophilic (optimal temperature for growth 20-40°C) some (e.g. certain *Cladosporium* species) are psychrophilic (optimum below 20°C) and cause serious problems in refrigerated food storage. *Aspergillus fumigatus* belongs to the other extreme, the thermotolerant fungi. This mould thrives in temperatures above 40°C and plays an important role in the

Table 1

Enzymatic ability of moulds commonly encountered in air- and house dust-analyses

Representative species	Attacked substances
<i>Alternaria alternata</i>	cellulose, pectin, starch, xylan
<i>Aspergillus versicolor</i>	pectin, xylan
<i>Botrytis cinerea</i>	cutin, cellulose, pectin
<i>Chaetomium</i>	xylan, cellulose, cellobiose
<i>Cladosporium herbarum</i>	lignin, lipids, pectin, cellulose, starch
<i>Mucor mucedo</i>	amylase, lipase
<i>Mucor spinosus</i>	pectin, chitin
<i>Mucor racemosus</i>	protein, hemicellulose, cellulose, pectin
<i>Penicillium expansum</i>	cellulose, pectin, xylan
<i>Penicillium frequentans</i>	cellulose, pectin
<i>Penicillium notatum</i>	protein, cellulose, xylan, pectin
<i>Phoma exigua</i>	xylan, pectin
<i>Phoma herbarum</i>	cellulose, xylan
<i>Scopulariopsis brevicaulis</i>	lignin, cellulose, xylan, chitin, protein-NH ₂
<i>Trichoderma viride</i>	cellulose, xylan, pectin (lignin)
<i>Ulocladium consortiale</i>	cellulose

[Domsch & Gams (9)].

economics as, like most of the thermophilic fungi, it is a cellulose decomposer attacking for example crops and hay which have been poorly stored.

pH. Compared with bacteria, fungi prefer more acid conditions, the optimal requirement for growth being pH 5–6. Probably the cells have an internal buffer-capacity during growth, as the endogenous respiration is almost unchanged during variation of the external medium at a pH range of 5–8.

The spore. The propagules of the fungi – different types of *spores* and *conidia* – correspond to the seeds in flowering plants. They range from 3–200 µm in size, most of them about 10 µm in diameter. The spores are often liberated into the air in massive concentrations and can remain airborne for a long time. The spore of the fungi is probably the major component causing the different

types of allergy since spores are inhaled and deposited on a sensitive mucosa. Gregory (13) has defined the fungal spore as the part of the fungus which is specialized for reproduction, survival and dispersal. Normally the spore is separated from the rest of the fungus and characterized by its minimal metabolic activity, low water content, and lack of cytoplasmic movements. In the spore a balance is established between protection against the environment and the ability to respond to variations in external stimuli (33).

The spore wall can be divided into one, two, or three layers: the outer, the exospore, which is a structure of rodlets, a middle layer and an inner layer where the active synthesis of the wall components takes place.

The chemical composition of the spore wall is still incompletely elucidated. The spores from *Mucor* and related genera contain glucan, polyglucosamine (chitin) and

melanin. 1–3% sporopollenin – the same component as found in pollen walls, is found in zygospores of *Mucor mucedo*.

Other studies have revealed that ungerminated spores contain components of both a protein and polysaccharide nature, e.g. t-RNA, aminoacyl-t-RNA, synthetase and transfer enzymes.

Some of the components are condensed on the surface of the spore and may be relevant to the allergic reaction. Small changes in certain components, e.g. mannans, are related to profound changes in the texture and shape of the spores (28, 33).

Liberation, dispersal and deposition of spores

Pepys (20) especially stresses the importance of environmental studies as a supplement to diagnosis and treatment of the allergic patient. A comprehension of the liberation, dispersal and final deposition of the spores is therefore essential. The major growth foci in nature are:

- (a) *the soil* where the fungi participate together with bacteria and actinomycetes in the organic decomposition processes. Another fungal activity in soil is the mutual interaction between higher plants and fungi, the latter utilizing root exudates, and supplying the plants with essential substances.
- (b) *the leaves* of trees and plants, where the so-called phylloplan fungal flora have been thoroughly studied. On growing beech leaves for example there is an exact fungal succession which starts with the deposition of spores of primary invaders such as *Sporobolomyces*, *Rhodotorula*, *Aureobasidium*, followed by secondary invaders such as *Epicoccum* and *Cladosporium* (8). The moulds do not start their decomposing activity until the autumn, when the leaves reach senescence. An excellent review of liberation mechanisms and dispersal is found in Ingold (15) and Gregory (13), but a few examples are given here: Spores

from *Mucor* can be liberated by turgor pressure, from *Botrytis* by hygroscopical movements, or from *Cladosporium* by mist-droplets, raindrop splash mechanism, or they can be blown off by the wind.

The spores are highly adapted for survival and dispersal. The wall protects against desiccation and is often pigmented as in the phylloplan fungi for example, making the fungi less vulnerable to radiation damage from the u.v. light in the atmosphere. After liberation, the spores are either transported into the air by microturbulence or other air currents, or they are forcibly ejected and dispersed over great distances, often hundreds of kilometers.

Before sedimentation and germination spores can be deposited on an allergic mucosa and thus release symptoms, or they can sediment indoors together with other house dust particles, be redispersed by indoor activities, or germinate in suitable places, e.g. moist basements, rooms with poor ventilation, or window frames with condensed water.

Mode of sensitization

Although the spores from mushrooms and toadstools as well as rust (*Puccinia*), smut (e.g. *Ustilago*) and dry rot (*Serpula lacrymans*) give allergic reactions, the most important fungal allergen sources are found among the saprophytic microfungi – the moulds – especially from the groups Zygomycotina (e.g. *Mucor*, *Rhizopus*) and the Deuteromycotina – Fungi imperfecti – (e.g. *Cladosporium*, *Alternaria* and *Aspergillus*) (Table 2).

A common feature of the moulds is their quick (a few days) and simple asexual reproduction-cycle resulting in the formation of enormous quantities of spores (conidia). The ability to quickly occupy and inhabit suitable ecological niches indoors as well as outdoors, and to form and disperse such masses of spores, perhaps explains their evolutionary success in nature and their role as allergen sources. People are exposed to

Table 2
Classification of fungi¹

I.	<i>Phycomycetes</i> (Zygomycotina) e.g. Mucorales "moulds", i.e. <i>Mucor</i> , <i>Rhizopus</i>
II.	<i>Ascomycetes</i> (Ascomycotina) e.g. Endomycetales "yeasts" i.e. <i>Saccharomyces</i>
III.	<i>Basidiomycetes</i> (Basidiomycotina) e.g. "mushrooms", toadstools, rust, smut, dry rot
IV. ²	<i>Fungi imperfecti</i> - "moulds" (Deuteromycotina)
	a) Moniliales
	1. Genera with conidia (spores) borne in basipetal succession. <i>Acremonium</i> , <i>Aspergillus</i> , <i>Fusarium</i> , <i>Gliocladium</i> , <i>Paecilomyces</i> , <i>Penicillium</i> , <i>Scopulariopsis</i> , <i>Stachybotrys</i> , <i>Trichoderma</i> , <i>Trichothecium</i> , <i>Verticillium</i> .
	2. Genera with blastospores, radulaspores, porospores and aleuriospores. <i>Acremoniella</i> , <i>Alternaria</i> , <i>Arthrinium</i> , <i>Aureobasidium</i> , <i>Botrytis</i> , <i>Drechslera</i> , <i>Graphium</i> , <i>Helminthosporium</i> , <i>Oedocephalum</i> , <i>Stemphylium</i> , <i>Ulocladium</i> , <i>Chrysosporium</i> , <i>Humicola</i> , <i>Microsporum</i> , <i>Trichophyton</i> , <i>Epidermophyton</i> , <i>Trichocladium</i> .
	b) Torulopsidales (yeast-like) <i>Rhodotorula</i> , <i>Candida</i> , <i>Trichosporon</i>

¹Classification of microfungi is difficult as they represent a much wider botanic spectrum than, e.g. the family: Grasses (Gramineae). The taxonomic criteria have been spore-similarity or similarity in spore-formation.

²The *Fungi imperfecti* are taxonomically an artificial group of microfungi with asexual reproduction (30).

heavy concentrations of spores at home, at work, or outdoors, which may result in sensitization in atopic individuals. For two of the common moulds, regarded as the main allergenic fungi, threshold concentrations for evoking allergic symptoms are estimated to be 100 *Alternaria* spores /m³ air and 3000 *Cladosporium* spores /m³ air. These concentrations are multiplied outdoors in late summer and autumn (3).

Prevalence of fungal allergy

The literature gives numerous examples of allergic manifestations after fungal contact. However, in cases where clinical data are supplied with studies on the patient's environment, revealing the fungal allergen sources, there is more certainty about which moulds actually cause the allergy.

Sensitivity to fungi was first mentioned by Floyer in 1726 (1). Blackley (1873) (1) described his own allergic reactions to spores

from *Chaetomium* and *Penicillium expansum*. For an inhalation-allergic population, prevalences ranging from 2–30% are suggested. Precise statements are, however, impossible to give, until clinicians have been furnished with highly purified, standardized diagnostic extracts.

The different diagnostic *in vivo* (skin test, nasal/bronchial provocation) and *in vitro* (RAST, histamine-release) tests, and the environmental parameters have to be combined in the allergological clarification. Cases exclusively based on skin tests with commercially available fungal allergen extracts are often less reliable because the tests are restricted to a standard repertoire dependent upon successful preparation procedures from the different manufactures and are not always in accordance with the moulds to which the patient is really exposed.

Environmental analyses from the individual patient help to identify the allergen

sources. Problems may arise because of the quality of the diagnostic extracts both for *in vivo* and *in vitro* tests.

Some commercially available fungal extracts have been dialysed for some years, e.g. the Danish extracts since 1974, which means that unspecific irritating components of low molecular weight that may raise false positive reactions are eliminated.

False negative reactions, e.g. where cutaneous and RAST reactions are not correlated to the obvious anamnestic information, may be explained partly by the fact that the extracts could contain many antigens irrelevant to the patient, and partly because the presence of precipitating antibodies against some of the mould antigens may result in a blocking of the IgE sites of the basophils.

Material used for fungal preparations

Spores and conidia from the microfungi are the main fraction inhaled by the patients, although mycelial fragments are found in pollen/spore traps as well.

As a consequence, fungal extracts have been prepared from cultures where the culture medium favours sporulation, from mycelial pellets containing spores, or from the biomass together with the consumed substrate. (14).

The allergenic components of the different culture fractions need further elucidation. Presumably the allergens are located on the surface of the cell wall and somatic (intracellular) substances may be avoided.

Variability of fungal extracts

Conservation of biochemical properties, and maintenance of trueness to type in the different strains, are problems well documented in the literature. Besides the cultivation conditions (which of course should be kept stable) factors such as: alterations of chromosomal number or structure, mitotic non-conformity, two or several nuclei (dual phenomenon), parasexual cycles, alterations due to several transfers

of inoculation material, and senescence (progressive strain deterioration), may influence the stability of antigenic components of the strains used (27, 28).

Immunochemical methods such as crossed immunoelectrophoresis (CIE) using specific rabbit antibodies raised against the moulds, and crossed radio immunoelectrophoresis (CRIE) using specific human serum from patients suffering from allergy to a given mould, are now applied to improve purity and standardize the extracts (Fig. 1A, B.) (18).

MANIFESTATIONS

Retention of particles

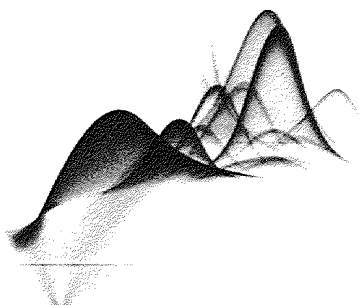
It is generally considered that particles $>5\mu\text{m}$ cause type I allergy and particles $<5\mu\text{m}$ cause type III. This is an oversimplification. If the patient is a nose breather, inhaled spores, depending on the condition of the patient, will be retained on the nasal mucosa. If the patient suffers from a nasal hindrance, he may be a mouth breather and particles up to $20\mu\text{m}$ may be deposited in the bronchioli (16). *Aspergillus fumigatus*, a common mould in the air and house dust, with spores of $3\mu\text{m}$ in size, provides a good example of a mould whose spores may cause either infection or a type I or III allergy.

The aetiological aspects of the genus *Aspergillus* have recently been reviewed by Pepys (20), but other airborne moulds may play a similar role.

Extrinsic allergic alveolitis is often linked to the thermophilic actinomycete *Micropholyspora faeni*, but mesophilic moulds from for example farm buildings, infected grain, or infected humidifiers, seem to play a certain role as evokers of type III reactions.

Type I. IgE-mediated reactions to common moulds are more established. Rhinitis and asthma (often in children) are the most common manifestations, but urticaria (e.g. after ingestion of blue cheese with *Penicillium*

A



B



Fig. 1. A. CIE (crossed immunoelectrophoresis) analysis of an antigen preparation of the grape mould *Botrytis cinerea* by means of specific rabbit antibodies raised against the mould.

B. Autoradiography of CRIE (crossed radio immunoelectrophoresis) demonstrating specific *Alternaria* allergens. Serum from an *Alternaria* sensitive patient was added to the crossed immunoprecipitates. (For methodological details, see (18)).

roqueforti), oedema (provoked for example by baker's yeast, *Saccharomyces cerevisiae*) and dermatitis are also recorded.

Diagnosis of type I and type III allergy caused by fungi

In contrast to pollen for example, a precise and reliable diagnosis of fungal allergy depends on several factors. As previously mentioned, the anamnestic information from the patient is very important, especially in connection with the environmental screening of his dwelling or work place.

Type I (inhalation allergy). Cutan test (preferably prick test) followed, if possible, by nasal or bronchial provocation. Good evidence is achieved if the *in vivo* diagnosis is followed by either RAST, CRIE or histamine-release.

Type III (extrinsic allergic alveolitis). Symptoms and causing agents in relation to, e.g. Farmers' lung or Malt workers' disease have been excellently treated by Pepys (19). In addition to the clinical picture exhibited by the patient (dyspnoea, cyanosis, fever, crepitation, diffuse lung infiltrates), the diagnosis can be supported by IgG analyses revealing specific precipitating antibodies. Current counter electrophoresis and crossed immunoelectrophoresis seem to be good tools for this purpose. In comparison with the Ouchterlony test, these methods seem to be more specific and sensitive, although further methodological studies are needed concerning quantification and references. The consequences of human antibodies to common moulds are incompletely studied and not fully understood, but demonstrations of specific precipitates to a given mould, isolated from the suspected allergen source, e.g. contaminated humidifiers, can be a valuable supplement to the diagnosis, revealing the specific exposition.

Caution must be used against misinterpretation caused by unspecific precipitin reactions to fungal somatic polysaccharides (6).

Final evidence may be obtained with a skin test leading to delayed reaction, or a provocation test followed by prolonged supervision.

Cross reactions between moulds

Allergenic specificity of the different moulds related to fungal allergy is currently under discussion. Some investigators favour species specificity, others support specificity on a generic level, still others propose group specificity.

Preliminary investigations with extracts of 14 different mould genera analysed against specific rabbit antibodies in crossed line immunoelectrophoresis revealed, however, very little partial identity (cross reaction) between the *antigens* of the 14 common moulds. Nonetheless, in relation to the antigens, we have experienced that on the species level, extended partial identity exists.

To finally solve this challenging problem, the major *allergens* in moulds must be demonstrated. Studies are in progress using

specific human sera in the extension, where it is possible to find patients with specific allergy to the moulds under investigation.

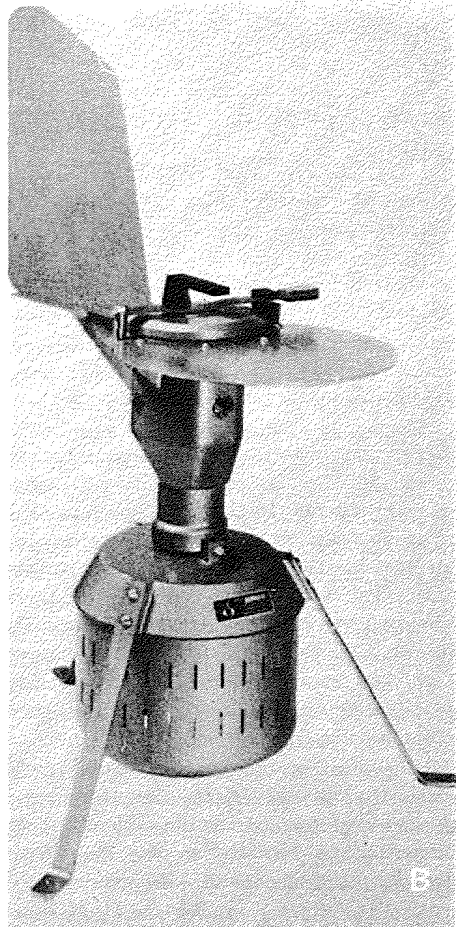
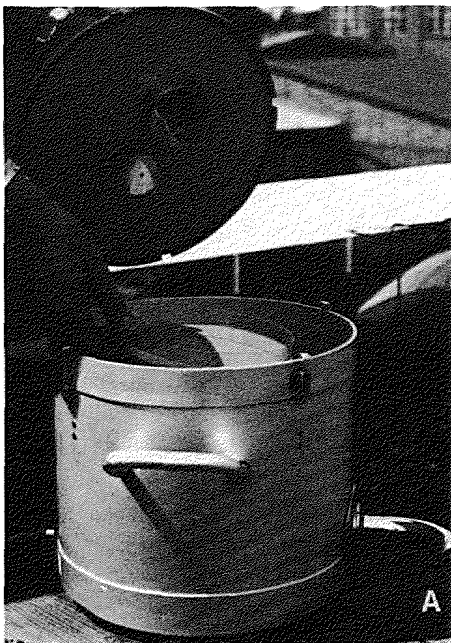
Environmental studies

Indoor/outdoor mould surveys. Owing to their ubiquitous presence in nature, fungi as an allergen source are almost inevitable. Consequently, investigations of the environmental conditions of dwellings, work places, and the surrounding neighbourhood must be an important part of allergy research (20).

Fig. 2. Registration methods for demonstration of airborne microfungi.

A. slit-sampler, *viable* method.

B. Burkard trap, *non-viable* method. Height: 1.2 m.



Seasonal changes in the patients' symptoms are thus connected with the seasonal variations of the moulds. From all parts of the world surveys of airborne outdoor spores have been performed (recently reviewed by Agarwal (1)) demonstrating the geographical and seasonal variations of the important fungi. These variations are obviously related to the variation of higher plants and climatic factors. The genus *Aspergillus*, for example, is more often encountered in the tropics than in temperate regions. A comprehensive and interesting discussion of the microbiology of the air is given by Gregory (13).

Outdoor spore registration can be performed in two fundamentally different ways (Fig. 2A, B): 1) methods which mainly favour the quantitative estimation where the *non-viable* and the indeterminate spores are included, and 2) methods based on cultivation with subsequent identification of the *viable* moulds. Advantages and disadvantages of the methods are discussed by Gregory (13).

Where it is practicable, registrations using the two different methods to supplement each other are preferred. In Denmark such dual surveys are performed and weekly spore bulletins issued.

Indoor registration. Demonstration and identification of indoor moulds may serve various purposes. In general, it gives an idea of which fungi the patients are exposed to. Seasonal variation with peaks in *late summer* and *autumn* (with the exception of *Penicillium* which is a *year-round* mould) is demonstrated. Although the fungi are encountered indoors it seems to reveal that for most of the moulds there is a certain relationship with the higher outdoor vegetation.

Demonstrations of the mould flora directly related to the patient's environment are routinely performed in our laboratory. Culture plates are sent to the homes or places of work of patients who are clinically suspected of having mould allergy. Results

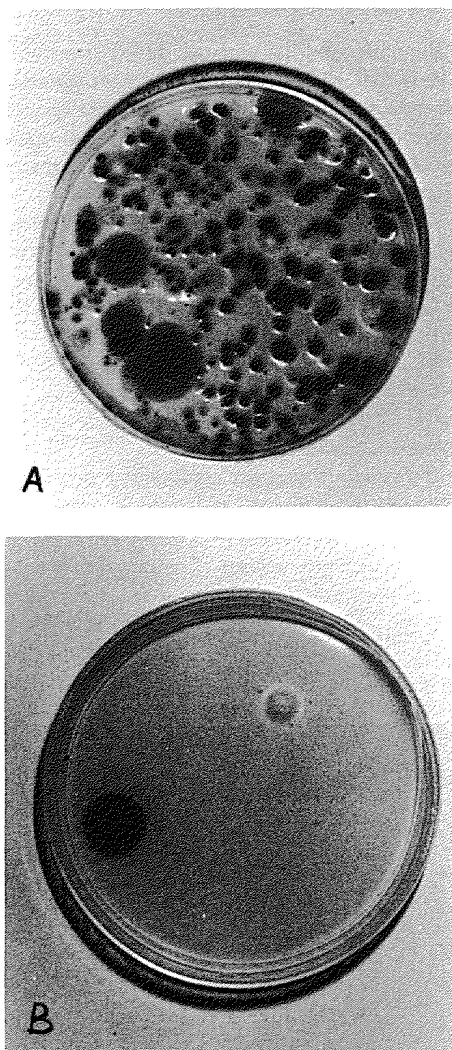


Fig. 3. Culture plates exposed for 20 min in a child's bedroom.

A. Mould colonies from settled spores before elimination.

B. Mould colonies after a thorough renovation of the room.

from the cultivation of settled spores from different places, as well as spores from a sample of house dust, reveal some of the microfungi to which the patient is exposed.

These results may be helpful when considering whether to renovate certain rooms in the home or place of work. Mould analyses may also serve as a test of the efficacy of environmental control at home or at work. "Much more commonly, environmental control should be employed as an adjunct to other forms of therapy including the use of hyposensitization and pharmacological agents". (20).

Whether the mould sensitive patient is subject to immunotherapy or not, elimination treatment is recommended, especially in the sleeping quarters, to provide the patient with 7–8 hours relief from fungal attacks.

Description of fungi related to allergic diseases

Selection of the fungi considered worthy of a more detailed description in this context depends upon the numerous indoor and outdoor surveys and clinical studies from different countries (1, 13, 21, 25, 29, 31). A common feature is their frequent occurrence in analyses of air and house dust (11, 12). Description of the fungi (2, 4, 9, 10, 27, 34) is restricted to features relevant to those who are not interested in botanical systematics but want some knowledge of the biology, ecology and occurrence of common moulds.

Attempts have been made to facilitate the diagnosis of fungal allergy by gathering together examples of places and materials which are most likely to be a risk for sensitive patients.

ALTERNARIA Neess ex Wallroth

50 species. Monographed by Neergaard, Joly and Simmons (2).

Diagnostic features: Colonies are grey, dark green, brown to black. The conidia characterize the genus. They are a shade of brown with transverse or longitudinal septae, often catenated and with a more or less pronounced beak. Size: 20–63 μm in length, 9–13 μm at the broadest part (*Alternaria alternata*). Other species have conidia up to 200 μm in length (Fig. 4A, B).

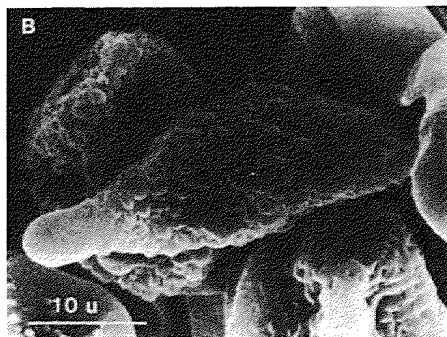


Fig. 4: *Alternaria*

A. Conidia seen in LM (light microscope) $\times 400$.

B. Conidium seen in SEM (scanning electron microscope) $\times 3000$.

Presence: Species of *Alternaria* are found everywhere, many as obligate parasites and as such unable to cultivate using conventional laboratory methods. *Alternaria alternata*, an extremely common saprophyte, is found on plants, both as a primary and a secondary invader of fruits and textiles. Black spots on tomato may originate from *Alternaria*. Frequently isolated from soil. Ability to decompose several sugars, cellulose, pectin and lignin.

Spores of *Alternaria* are considered to be connected with Baker's asthma. *Alternaria* is often found growing together with *Cladosporium* and *Aureobasidium* (Pullularia) on window frames either because of poor room ventilation with subsequent formation of condensed water, or because the window frames are badly fitting.

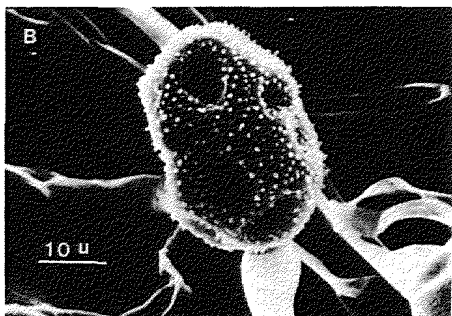


Fig. 5.
A. *Helminthosporium*. Conidia seen in SEM $\times 600$.
B. *Stemphylium*. Conidium seen in SEM $\times 1800$.

Closely related genera: *Curvularia* ($7 \times 20 \mu\text{m}$) very seldom recorded in Scandinavia, *Helminthosporium* ($30 \times 100 \mu\text{m}$) (Fig. 5A), *Ulocladium* ($12 \times 19 \mu\text{m}$) (*Pseudostemphylium*) and *Stemphylium* ($25 \times 40 \mu\text{m}$) (Fig. 5B). They are all of frequent occurrence, but are difficult to maintain in laboratory cultures. They often become irreversibly sterile after a few transfers (26).

ASPERGILLUS Micheli

About 160 species. The genus is described in detail by Raper & Fennell (22), with a chapter on pathogenicity by Austwick.

Diagnostic features: Colonies are all shades of green, e.g. bright green (*Aspergillus glaucus*), yellow green (*Aspergillus flavus*), greyish green (*Aspergillus fumigatus*). Or all shades of brown, e.g. black brown (*Aspergillus niger*), tan (*Aspergillus terreus*), yellow (*Aspergillus ochraceus*); or white (*Aspergillus candidus*).

The conidiophores (the spore-bearing organs) are erected on the mycelium. One elongated cell which swells to a head, with bottle-shaped phialides producing the asexual spores – the conidia – in chains. (Fig. 6).

The size of the conidia ranges from $3\text{--}7 \mu\text{m}$. They are single-celled, hyaline to bright coloured, spherical, smooth to echinulate.



Fig. 6. *Aspergillus fumigatus*, conidiophore with phialides and conidia. SEM $\times 1800$.

Presence: The genus is cosmopolitan, grows well at 20–26°C. Thermophilic species (e.g. *Aspergillus fumigatus*) exist with an ability to grow at temperatures up to 50°C. It is isolated from hay compost and is able to infect warm-blooded animals, including man.

The genus is frequently recorded in *tropical* and *temperate soil* and found on *plants* and *plant debris*.

Members of the *Aspergilli* are isolated from raw textile materials, e.g. cotton, kapok, hemp and jute.

As some people have positive skin reactions to untreated textile materials, the primitive conditions under which jute fibres for example are produced in Bangla Dsh should be noted. Jute plants are processed for manufacture by so-called "wet retting". The stalks are simply thrown into the water, where the soft plant parts are attacked and decomposed by various microorganisms including *Aspergillus* and e.g. *Chaetomium*.

Different species of *Aspergillus* possess a broad spectrum of enzymes, e.g. pectinase, xylanase, amylase, protease, amino-glucosidase. Concerning the risks for occupational allergy, examples of the industrial application of *Aspergillus* and other genera are shown in Table 3.

Production of citric acid by *Aspergillus niger* is estimated to more than 90 million kg/year, which proves this extremely common mould to be an important organism economically.

Special care must be taken regarding the

industrial use of fungi commonly found in people's normal environments, because sensitization at the place of work may extend to the patients' private environment.

Besides the aspects regarding allergy, increasing attention is being paid to low molecular (mol.wt. < 500) fungal metabolites from e.g. *Aspergillus flavus* (aflatoxin) and *Aspergillus ochraceus* (ochratoxin). Studies of mycotoxins have revealed that these components, which are excreted in mouldy fodder, are nephro- or hepatotoxic to domestic animals fed with such foodstuffs. Amounts of 1 µg/kg are known to have carcinogenic effects on rats.

Members of *Asperigillus* may act as opportunistic pathogens infecting lungs, ears, and eyes (e.g. *Asperigillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*).

Aspergillus is linked with e.g. Farmer's lung, Baker's asthma, and Maltworker's diseases.

The different clinical manifestations of *Aspergillus*: Bronchial asthma, allergic broncho-pulmonary aspergillosis, extrinsic allergic alveolitis, Aspergilloma and invasive aspergillosis, are reviewed by Pepys (20).

AUREOBASIDIUM Viala & Boyer

2–3 species, earlier named *Pullularia* or *Dematium*. *Aureobasidium pullulans*, the most common species, is treated in a monograph by Cooke (5).

Table 3

Examples of the industrial applications of some microfungi

Industrial role	Representative species
Production of antibiotics	<i>Penicillium notatum</i> , <i>P. Chrysogenum</i> , <i>P. patulum</i>
Production of enzymes	Bread-making industry: <i>Aspergillus oryzae</i> , <i>A. niger</i> , <i>A. awamori</i> Beer-making industry: <i>Aspergillus niger</i> Cheese-making industry: <i>Mucor mihei</i> Paper-making industry: <i>Aspergillus niger</i>
Transformation of steroids	<i>Aspergillus ochraceus</i> , <i>Fusarium solani</i>
Transformation of antibiotics	<i>Aspergillus ochraceus</i>
Production of organic acids	Citric acid: <i>Aspergillus niger</i> , <i>A. terreus</i>

(Smith & Berry (28))

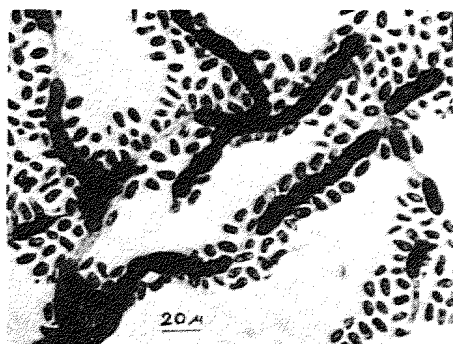


Fig. 7. *Aureobasidium pullulans*. Thick-walled, dark mycelium and conidia. LM $\times 450$.

Diagnostic features: At the beginning, this fungus develops white to cream-coloured yeast-like colonies. Later on they become more or less black and slimy. The mycelium is mostly immersed together with hyaline, ovoid singled-celled conidia (usually $5 \times 3 \mu\text{m}$) produced in slimy masses. The mycelium from which the spores are derived on denticles becomes more and more pigmented and thick-walled with age. (Fig. 7).

Presence: *Aureobasidium* is known as a primary invader of all kinds of leaves. During the summer the spores are deposited on the leaf surface without attacking the cells. In autumn, however, when the leaves reach senescence, *Aureobasidium* starts its pectolytic activity, decomposing the middle layer of the cell walls, which consist of pectic components. This attack disintegrates the leaves and facilitates the decomposing activity of secondary invaders such as *Cladosporium herbarum* which attacks e.g. the cellulose components in the cell wall.

Aureobasidium is often found in kitchens and bathrooms and can damage interior painted surfaces. Found on the honeydew from aphids.

BOTRYTIS Micheli ex Fries

30 species comprising several plant pathogens.

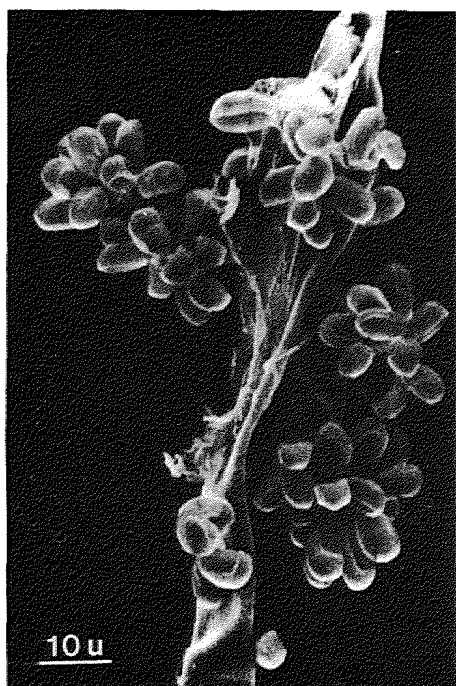


Fig. 8. *Botrytis cinerea*. Clusters of conidia. SEM $\times 1200$.

Diagnostic features: The colonies are more or less effuse, grey to brownish often with a characteristic "mould-like" odour. Seen in dissection microscope, the conidiophores look like "flowering cherry trees" with the stout brown conidiophores bearing pale glistening conidia (usually $11 \times 8 \mu\text{m}$). The latter are produced on short denticles, single-celled, hyaline or pigmented, spherical to ovoid. The spores are liberated by hygroscopical movements of the conidiophores (Fig. 8).

Presence: *Botrytis* is found on a wide variety of plant material in particular. Common species are *Botrytis cinerea* and *Botrytis allii*. The genus is especially seen in connection with soft fruits, e.g. strawberries and grapes. Growth of *Botrytis cinerea* on wine grapes is regarded as giving an added effect to the bouquet of certain wines.

Botrytis allii can seriously deteriorate *onion-*loads during transportation.

Botrytis cinera decomposes glucose, pectin, cellulose and cutin.

Pectolytic enzymes from *Botrytis* are used industrially in the production of marmalade.

CHAETOMIUM Kunze ex Fries

About 100 species. Macroscopically visible fruit-bodies (perithecia) are produced after a sexual process and surrounded with stiff, often branched, dark hairs. The spores (ascospores) are often liberated in their spore sacs (asci).

Presence: *Chaetomium globosum*, the most frequently encountered species, is a cosmopolite and has been isolated from plant compost, paper and other material containing cellulose. The mould possesses a strong cellulolytic activity. Other enzymatic ability: degradation of sugars, starch and cellulose. Heavy growth on sterilized straws inoculated with *Chaetomium* spores. Isolated from fibres of *jute* and *hemp* (see presence of *Aspergillus*).

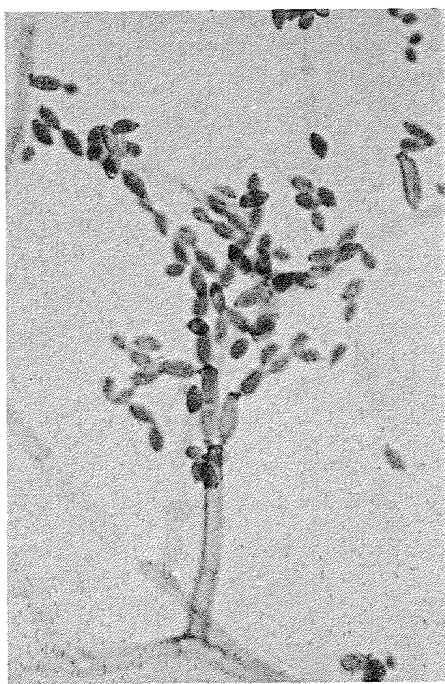
Thermophilic species (*Chaetomium thermophile*) and coprophilous species (e.g. *Chaetomium olivaceum*) exist, the latter may cause problems in mushroom cultivation.

CLADOSPORIUM Link ex Fries

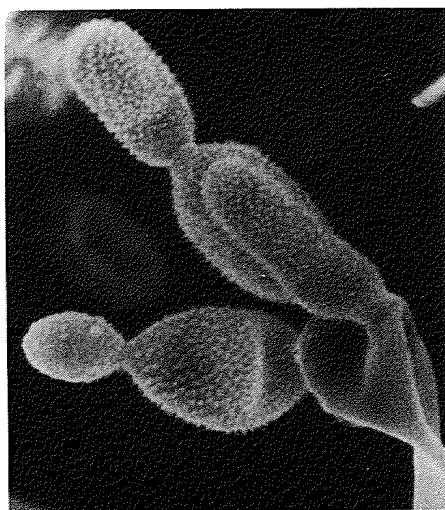
About 25 species described in detail by de Vries (32), earlier known as *Hormodendron* or *Hormodendrum*.

Some 500 species have been ascertained, perhaps due to the altered morphology of a given species in response to altered external conditions, a feature often found among the Fungi imperfecti.

Diagnostic features: The colonies are low, dense, often a velvety olive green, but also other shades of brown, dark or greyish green



A. *Cladosporium herbarum* seen in LM x 400.



B. *Cladosporium* sp. seen in SEM x 3000.

Fig. 9. *Cladosporium*

are seen. The erect pigmented conidiophores have tree-like branchings, the conidia are multi-shaped and show considerable variations in size and septation. Two characteristic spore forms are produced in *Cladosporium herbarum*: Lemon-shaped conidia, e.g. ($5 \times 3 \mu\text{m}$) and torpedo-shaped, e.g. ($23 \times 8 \mu\text{m}$) (Fig. 9A).

Presence: The genus is ubiquitous and especially abundant in temperate regions as a secondary invader of dead plants and trees. The dry spores produced in excessive quantities can be transported over wide areas, even oceans. Recordings from all parts of the world show that with a few exceptions *Cladosporium* is the most frequently encountered mould in air. *Cladosporium herbarum*, *Cladosporium cladosporioides*, *Cladosporium macrocarpum* (Fig. 9B), are all common species. Temperature optimum ranges from $18\text{--}28^\circ\text{C}$ but growth down to -6° is possible.

Plate counts from beech wood (*Fagus*) or from houses situated near beech trees often show considerable quantities of *Cladosporium* colonies. Frequently isolated from soil, foodstuffs, paints and textiles.

Growth of *Cladosporium* is frequently found in uncleaned refrigerators or on moist window frames, where they are able to cover the whole painted area. *Cladosporium* have even been isolated from tight window frames around thermo-windows, presumably due to formation of condensed water.

Houses with poor air ventilation, new as well as older ones, houses with a straw roof, and homes situated in low damp environments, often have heavy concentrations of *Cladosporium* spores expressed in high plate-counts after domestic analyses.

Cladosporium resinae is frequently isolated from the fuel tanks of aircrafts, and can be a nuisance to the petrol industry. It is found on creosoted timber and isolated from some face creams.

Cladosporium attacks cellulose, pectin and lignin.

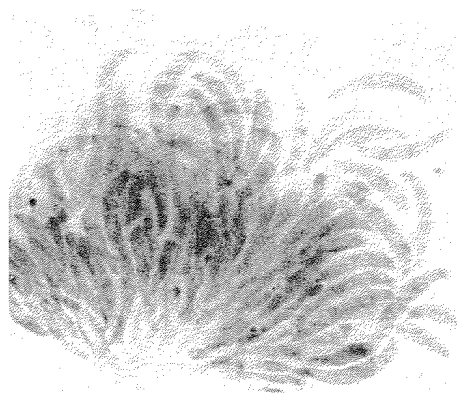


Fig. 10. *Fusarium* Typical conidia produced in cushion-like masses (sporodochium). LM $\times 400$.

FUSARIUM Link ex Fries

46 species.

Diagnostic features: The different species often form brightly coloured colonies, e.g. pink, yellow or apricot. The genus is readily recognized by the hyaline gondola-shaped septed macroconidia, produced in cushion-like masses (sporodochia). Identification of the many species is difficult as there is great heterogeneity in connection with different culture procedures (Fig. 10).

Presence: *Fusarium* is a very common soil fungus. Many species cause serious plant diseases. *Fusarium* is often isolated from barley. In the brewery industry, *Fusarium* can cause much trouble by producing excessive froth. Mycotoxins from many species of *Fusarium* are known to cause problems in cattle fed with mouldy grain. Several industrial applications.

MUCOR Micheli

The genus is reviewed by Zycha et al. (34) and consists of about 40 species.

Diagnostic features: Colonies are often white to greyish with large – up to centimeter-tall

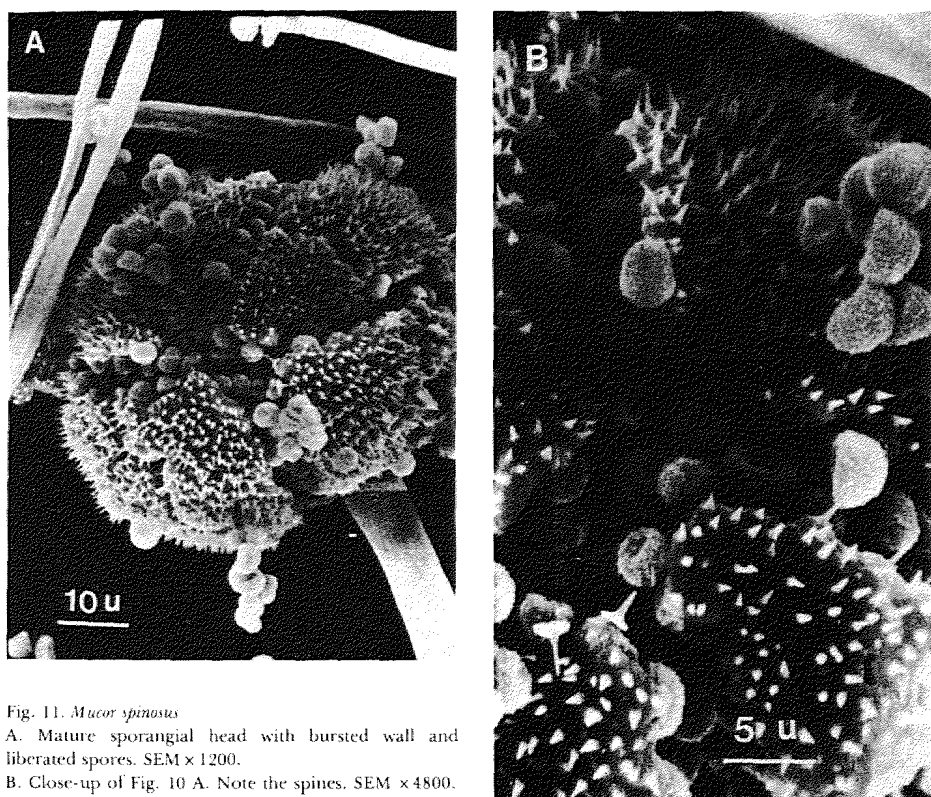


Fig. 11. *Mucor spinosus*

A. Mature sporangial head with bursted wall and liberated spores. SEM $\times 1200$.

B. Close-up of Fig. 10 A. Note the spines. SEM $\times 4800$.

needle-like sporangiophores with a terminal sporangium in which asexual spores are developed. At maturity the sporangial wall is broken and the spores released in abundance. The cell wall consists of chitin-chitosan components. The sporangiospores – 3–15 μm in size – are round or oval, hyaline to brownish, smooth to rough (Fig. 11A, B).

Presence: The genus *Mucor* belongs to the “sugar fungi”, which quickly inhabit and decompose the carbohydrates available, e.g. on dead plant material, horse dung, in soil, or on rotting mushrooms. Species of *Mucor* are often seen in the household, e.g. on soft fruit, fruit-juice and marmalade. In the heavy fraction of house dust, the so-called floor-dust, *Mucor* is the dominating mould.

Common species: *Mucor racemosus* (minimum humidity for growth: 92%, for sporulation 95%) isolated from soil, horse dung, plant material and sour milk.

Mucor spinosus (plumbeus): House dust, air.

Mucor mucedo: Dung, plant material, butter, cheese, meat and leather material.

Mucor pusillus: A thermophilic species often found in house dust inoculated at 37°C. Isolated from horse hair and dandruff, cow hair and dandruff and jute (see p. 11).

Enzymatic ability: Different *Mucor* species are able to attack starch, lipids, pectin, protein, chitin, hemicellulose and cellulose.

Industrial applications: The so-called rennet protease, produced from *Mucor mihei*

and *Mucor pusillus*, plays an increasing role in cheese production as a substitute for natural rennet.

Species of *Mucor* are also used in the production of fruit pulp and juice. Their proteolytic enzymes work at low pH optimum which makes them suitable in the breakdown of fruit pectins.

Infections in domestic animals caused by *Mucor* are recorded, (mucormycosis).

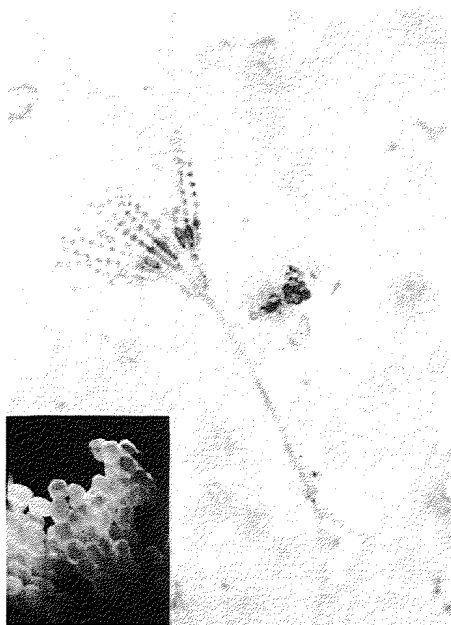


Fig. 12. *Penicillium* Pencil-like conidiophore. LM $\times 400$. Insert. Conidial chains. SEM $\times 2000$.

PENICILLIUM Link ex Fries

About 220 species reviewed by Raper & Thom (23). The genus has been intensively studied because of the economically important antibiotic-producing species.

Diagnostic features: Colonies are mostly shades of dusty green. The conidiophores have characteristic pencil-like spore-bearing organs (phialides) from which the small

round hyaline conidia (2–5 μm) are abundantly produced in long chains (Fig. 12).

Presence: Like the *Aspergilli*, members of the *Penicillia* are omnipresent saprophytes. The genus is dominating in temperate soils, and the spores are easily liberated and dispersed into the air. In contrast to most other common moulds, *Penicillium* has no indoor seasonal variation, but an outdoor maxima in the spring and winter, both in town and country. Because of a pronounced variable enzymatic ability, different species can be isolated from almost all organic materials.

Members of *Penicillium* are frequently found on stale bread, citrus fruits (*Penicillium italicum*) and apples (*Penicillium expansum*).

In degradation of fruits and legumes for example, *Penicillium expansum* produces a mycotoxin – patulin – a low molecular substance (mol.wt. 100–200 daltons) which has carcinogenous effects on liver, lung, and brain.

Penicillium associated with e.g. rye flour is a problem causing after-contamination of bread in industrial bakeries.

Secondary metabolites from members of *Penicillium* have been revolutionary in combating infectious diseases such as pneumonia and gonorrhoea.

By chance, Alexander Fleming observed that a colony of a contaminating *Penicillium notatum* in a petri dish had a hampering effect on the *Staphylococcus aureus* colonies inoculated on the agar surface. This observation led to the discovery of the potent antibiotic called Penicillin.

Systematic screenings of other soil fungi with the ability to produce similar secondary metabolites with antibacterial effects have been done for many years, but most of the antibiotics found are too toxic for practical use. Besides *Penicillium notatum*, *Penicillium chrysogenum* is a good producer of penicillin. A connection between allergy to the metabolite penicillin and the mould *Penicillium* is not yet shown.

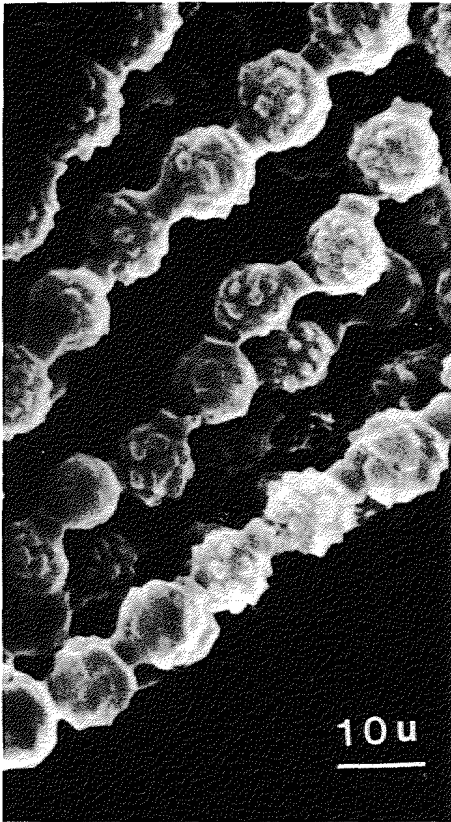


Fig. 13. *Scopulariopsis*. Note the warty conidia. SEM $\times 1200$.

Other important species are *Penicillium roqueforti* and *Penicillium camemberti* used in the production of blue cheese and camembert cheese.

Many species of *Penicillium* play an important industrial role in the transformation of organic components and the production of enzymes and antibiotics.

Closely related morphologically to *Penicillium* are the two genera *Paecilomyces* (Spicaria) and *Scopulariopsis*. They mostly exhibit shades of brown although green *Paecilomyces* exist. *Scopulariopsis* has oval truncate conidia with a basal rim, often verrucose (Fig. 13).

Both genera are omnipresent. *Scopulariopsis*



Fig. 14. *Phoma* bowl-shaped fruitbody with spores in slimy masses protruding from the apex. LM $\times 64$.

brevicaulis, the most common species, may attack *fungernails*. *Scopulariopsis* is often isolated from *foodstuffs*, *paper*, *sewage*, *soil*, *textiles* and *plant material*. Found in *guinea pig hair*, *cat hair* and *cow hair*.

PHOMA, sensu Sacc.

70 species.

Diagnostic features: The genus is characterized by bowl-formed fruitbodies (pycnides). The conidia pressed through the aperture in slimy masses are hyaline and single-celled (Fig. 14).

Presence: *Phoma* is a very common *soil fungus* attacking weak or damaged plants. Frequently isolated from *potatoes* (*Phoma exigua*). Like *Cladosporium* and *Aureobasidium*, *Phoma* is described in connection with biodeterioration of *painted walls*, producing coloured spots often pink or purple, several centimeters in diameter.

RHIZOPUS Ehrenberg

Closely related botanically to *Mucor* and inhabiting same ecological niches. 12 species reviewed by Zycha et al. (34).

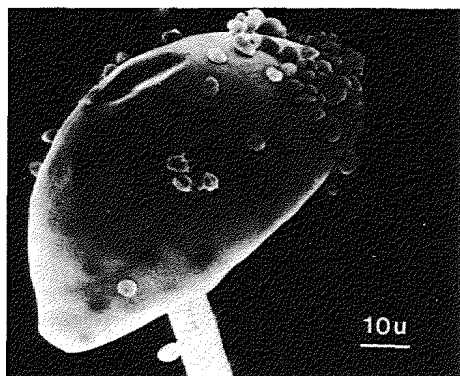


Fig. 15. *Rhizopus*. Mature sporangial head with spores remaining on the collapsed columella. SEM $\times 1200$.

Diagnostic features: The centimeter-tall grey colonies with needle-like sporangiophores with black heads are easily recognized and distinguished from colonies of *Mucor* by the presence of brown root-like hyphae (rhizoids). (Spores $9\text{--}11\text{ }\mu\text{m}$).

Presence: Often found in *kitchens* on forgotten left-overs of *stewed fruit*.

In the laboratories *Rhizopus* can be a serious contaminator of cultures because of the abundant spore liberation and extensive growth.

The species *Rhizopus nigricans* is the most common mould of the Mucorales. *Rhizopus* is used in the production of proteases and amyloglucosidases, lipases, rennet proteases, and pectinases.

RHODOTORULA Harrison

11 species.

Yeast colonies with orange, pink or yellow pigments. One of the most common airborne organisms. Frequently found on leaves of *potted plants*.

TRICHODERMA Persoon ex Fries

10 species reviewed by Rifai (24).

Diagnostic features: Colonies of *Trichoderma viride*, the most common species are white to

bright green. The colonies grow in a characteristic effusion and often a smell of coconut aids identification of the fungus. Conidia are very small ($2\text{--}3\text{ }\mu\text{m}$) single-celled, oval, produced in slimy clusters.

Presence: *Trichoderma*, the most widespread soil fungus isolated from numerous different geographical locations. It can grow on other fungi and is commonly found on *fallen timber*. Commonly isolated from coniferous needles (spruce and fir). Its strong cellulolytic activity facilitates growth on *damp timber* and *paper*. It is found on *tapestry*, in *moist dwellings* and often isolated in *kitchens*, where it grows on *unglazed ceramics*.

Besides the ability to attack cellulose, it may decompose starch, pectin, chitin and lignin. Several potent antibiotics are produced by *Trichoderma viride*, but unfortunately they are too toxic for human use.

In the industrial cultivation of mushrooms, *Trichoderma* can cause some problems. Tulip bulbs may frequently be infected by *Trichoderma*.

TRICOTHECIUM Link ex Fries

One common species, *Trichothecium roseum*.

Diagnostic features: Pink powdery colonies. Conidiophores with two-celled conidia ($5 \times 13\text{ }\mu\text{m}$), resemble golfclubs in appearance.

Presence: Found in e.g. *stored apples*, on *hazelnuts* and *chestnuts*.

VERTICILLIUM Nees ex Wallroth

30 species.

Diagnostic features: Colonies white to pigmented. *Verticillium lateritium* with a characteristic brick-red colour. Conidiophores often in typical branch arrangement. Conidia are produced in a mucous which turns to slime and forms into balls at the apex.

Presence: Very common in soil, on dead wood and bark. Many are plant parasites. Decompose chitin and cellulose.

CONCLUSION

For several reasons the aetiological role of fungi in relation to allergic diseases is far from being completely elucidated. One of the difficulties concerns identification, both at the generic- and species level, especially in connection with air analyses using "non viable" methods. Questions concerning cross reactivity (partial identity) between the genera and species of the fungi, variability of the extracts, and components irrelevant to the demonstration of a specific allergy are still a challenge, especially to the "immunocrochats" (Aas). Adequate tools are for example specific rabbit antibodies and human sera applied to RAST, RAST-inhibition, QIE (quantitative immunoelectrophoresis), CCIE, (counter current immunoelectrophoresis), and CRIE (crossed radio immunoelectrophoresis); the same methods employed in the identification, characterization and isolation of the allergenic molecules. (18).

As common moulds are subjected to an increasing industrial exploitation, the protection of people likely to incur fungal exposition must be extremely effective, because sensitization from the applied moulds, eventually leading to occupational allergy, may extend to the patients' home environment where the same moulds are found.

Isolation and identification of microfungi from the environment of the patient as well as demonstration of specific IgE (type I allergy) or demonstration of specific IgG (type III allergy) confirm the specific exposition and are recommended partly as a supplement to the diagnosis, and partly as a guidance in elimination treatment.

ACKNOWLEDGEMENTS

The Botanical Institutes, University of Copenhagen, are gratefully acknowledged for placing their Scanning

Electron Microscope at my disposition. I am grateful to lecturer, Jørgen Kristiansen, B.A., Institute of Plant Anatomy and Cytology, for helpful advice during the SEM-operations. Lisbeth Larsen, B.Sci., is thanked for inspiring discussions during the work.

The skilful technical assistance of Jane Piasecki, Jørgen Jacobsen, and Inger Borberg is appreciated. I am grateful to Robert Mortensen for revising the English.

This work was supported by Allergologisk Laboratorium and Diagnoselaboratoriet, Copenhagen.

REFERENCES

1. Agarwal, K. K. & Shivpuri, D. N.: Fungal spores - their role in respiratory allergy. In Nair, P. K. K. (ed.): *Advances in pollen - spore research*. Vol. 1, pp. 78-128, 1974.
2. Arx, J. A. von: The genera of fungi sporulating in pure culture. J. Cramer. Hannover, Münden, 1970.
3. Bagni, N., Davies, R. R., Mallea, M., Nolard, N., Spiessma, F. T. & Stix, E.: Sporenkonzentrationen in Städten der Europäischen Gemeinschaft (EG). *Acta Allergol.* 32, 118-138, 1977.
4. Barron, G. L.: The genera of hyphomycetes from soil. The Williams and Wilkins Co. Baltimore, 1968.
5. Cooke, W. B.: An ecological life history of *Aureobasidium pullulans* (de Bary) Arnaud. *Mycopathologia* 12, 1, 1959.
6. Davies, R. J. & Pepys, J.: Occupational asthma. In Clark, T. J. H. & Godfrey, S. (eds.): *Asthma*, chapter 10. Chapman & Hall. London, 1977.
7. Dennis, C.: The microflora of the surface of soft fruits. In Dickinson, C. H. & Preece, T. F. (eds.): *Microbiology of plant surfaces*. Academic Press, London, 1976.
8. Dickinson, C. H.: Fungi on aerial surfaces of higher plants. In Dickinson, C. H. & Preece, T. F. (eds.): *Microbiology of plant surfaces*. Academic Press, London, 1976.
9. Domsch, K. H. & Gams, W.: *Pilze aus Agrarböden*. G. Fischer Verlag, Stuttgart, 1970.
10. Ellis, M. B.: *Dematiaceous hyphomycetes*. Commonwealth Mycological Institute, Kew, 1971.
11. Gravesen, S.: Identification and quantitation of indoor airborne microfungi during 12 months from 44 Danish homes. *Acta Allergol.* 27, 337-354, 1972.
12. Gravesen, S.: Identification and prevalence of culturable mesophilic microfungi in house dust from 100 Danish homes. *Allergy* 33, 268-272.
13. Gregory, P. H.: *The microbiology of the atmosphere*. Leonard Hill, Aylesburg, 1973.
14. Hyde, H. A.: Atmospheric pollen and spores in relation to allergy. I & II. *Clin. Allergy* 2, 153-179, 1972 and 3, 109-126, 1973.
15. Ingold, C. I.: *Fungal spores, their liberation and dispersal*. Clarendon Press, Oxford, 1971.

16. Jonassen, N. (ed.): *Termisk og atmosfærisk indeklimate*. Polyteknisk Forlag, Lyngby, 1970.
17. Lustgraaf, v. d. B.: Ecological relationships between microorganisms and house-dust mites (Acarida : Pyroglyphidae). (Thesis). Stichting, Studentenpers, Nijmegen, Holland, 1978.
18. Lowenstein, H.: Quantitative immuno-electrophoretic methods as a tool for the analysis and isolation of allergens. *Prog. Allergy* 25, 1-62, 1978.
19. Pepys, J.: Hypersensitivity diseases of the lungs due to fungi and organic dusts. *Monographs in Allergy*. Vol. 4, S. Karger, Basel, New York, 1969.
20. Pepys, J. & Davies, R. J.: Allergy. In Clark, T. J. & Godfrey, S. (eds.): *Asthma*, chap. 7. Chapman & Hall, London, 1977.
21. Prince, H. E. & Meyer, G. H.: An up-to-date look at mould allergy. *Ann. Allergy* 37, 18-25, 1976.
22. Raper, K. B. & Fenell, D. I.: *The genus Aspergillus*. The Williams & Wilkins Co., Baltimore, 1965.
23. Raper, K. B. & Thom, C.: *A manual of the Penicillia*. The Williams & Wilkins Co., Baltimore, 1949.
24. Rifai, M. A.: A revision of the genus *Trichoderma*. *Mycol. Pap.* 116, 56, 1969.
25. Ripe, E.: Mould allergy (Thesis), Sweden, 1966.
26. Simmons, E. G.: Typification of *Alternaria*, *Stemphylium* and *Ulocladium*. *Mycologica* 49, 529-533, 1967.
27. Smith, G.: *An introduction to industrial mycology*. E. Arnold, London, 1971.
28. Smith, J. E. & Berry, D. R.: *The filamentous fungi*. E. Arnold, London, 1975.
29. Solomon, W. R.: Assessing fungus prevalence in domestic interiors. *J. Allergy Clin. Immunol.* 53, 235-242, 1975.
30. Talbot, P. H. B.: *Principles of fungal taxonomy*. MacMillan Press, London, 1971.
31. Van der Werff, P. J.: *Mould fungi in bronchial asthma*. H. E. Stenfert Kroese, N. V. Leiden, 1958.
32. Vries, C. A. de: Contribution to the knowledge of the genus *Cladosporium* Link ex Fr. (Thesis). Baarn, 1952.
33. Weber, D. J. & Hess, W. M. (eds.): *The fungal spore*. Wiley & Sons, New York, 1976.
34. Zycha, H., Siepmann, R. & Linnemann, G.: *Mucorales*, J. Cramer, Hannover, Münden, 1969.

Address:

Suzanne Gravesen
 Allergologisk Laboratorium
 Ved Amagerbanen 23
 2300 Copenhagen 5
 Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.