# FUNGI
## A KINGDOM OF THEIR OWN

The inconspicuous fruit bodies of ***Dasyscyphus virgineus*** are only about 1 mm in diameter.

Although we may not always realise it, fungi and their products are part of our daily lives. We consume fungi in all sizes, shapes and colours. Fungi not only include mushrooms and toadstools, but also yeasts and moulds. Yeast and moulds are used for the production of bread, cheese, salami, wine, beer or industrial alcohol. Fungi such as the death cap or fly agaric may produce highly toxic poisons via their secondary metabolism, but secondary metabolites may also be useful to medicine as ***antibiotics*** and ***immunosuppressant*** drugs. Without these drugs many human infections by pathogenic bacteria would take a deadly course, and organ transplantations would result in graft rejections by the recipient patient's immune response.

Fungi may be cultivated in large bioreactors for the production of vitamins, enzymes for washing-powder, or flavours and aromas. Fungi may be grown on renewable raw materials and have been the foundation, together with bacteria, for processes of ***industrial biotechnology*** for decades. Surprisingly, new species of fungi are continually being discovered, thereby enhancing our research options and unlocking new research avenues.

The mushroom-type fungus *Strobilurus tenacellus* is the original producer of the strobilurins, template for an important group of agricultural fungicides.

Spore-bearing structure (conidiophore) of the very strain of *Penicillium notatum* originally grown by Sir Alexander Fleming. Penicillin was first isolated from this fungus.

Fungi and their products serve an unusually broad spectrum of applications. This may be explained by their way of life and their habitats: ***Taxonomically*** speaking, fungi are neither plants nor animals but belong to a kingdom of their own. They may be found in all habitats and ecological niches, and some of them can cause deadly infections in other organisms, be they human, animal or plant.

### What are fungi?

As ***heterotrophic organisms***, fungi have a nutrition which is dependent on organic material produced by other organisms (especially plants). In order to achieve a thorough colonisation of such organic substrates, fungi grow as very fine thread-like ***hypha***e, collectively called mycelium, which infest the substrate. Enzymes are excreted in order to degrade polymers and release their monomers as carbon and nitrogen sources. The hypha facilitates variations around the heterotrophic theme. Thus, fungi have acquired several different functions in ecosystems: they may be ***saprotrophs***, ***parasites*** of other organisms or symbionts of plants or animals.

Whereas many fungi grow as minute moulds best seen with the microscope, in others hyphae aggregate to form larger structures visible to the naked eye as fruit bodies. Among these fruit bodies are the mushrooms and toadstools which may be collected in woodlands and used for culinary purposes. Fungi display large variations in morphol-

**Antibiotics**
Substances, mostly of microbial origin, which may kill bacteria (bactericidal action) or retard their reproduction (bacteriostatic action).

**Heterotrophic organisms**
Organisms which are not autonomous in their carbon nutrition but dependent upon external organic carbon sources (mostly other organisms).

**Hypha**
Thread-like growth form of fungi; the entire network of hyphae of a fungus is called mycelium.

**Immunosuppressants**
Substances which suppress the human immune system.

**Industrial biotechnology**
Biotechnological production of bulk and fine chemicals, enzymes, food and feed additives, pharmaceuticals and agrochemicals, auxiliary materials for processing industries etc.

**Taxonomy, taxonomic**
The classification of organisms into groups (taxa). In phylogenetics, organisms are placed on a phylogenetic tree of life which represents ancestry and relationships of all organisms.

*Trametes versicolor*, a typical white-rot fungus of wood.

**Algae**
Uni- or multi-cellular organisms capable of photosynthesis but not belonging to the plants. Algae include certain protist groups such as diatoms as well as red, brown and green algae.

**Cyanobacteria**
Capable of oxygen-releasing photosynthesis, these were formerly regarded as blue-green algae. However, they do not possess a true nucleus and therefore belong to the prokaryotes.

**Mycorrhizal fungi**
Mycorrhizal fungi are symbiotic endophytes and live in association with plants. They provide minerals to the plant, in turn receiving carbon compounds as nutrition which they cannot synthesise themselves. Some fungi enter endosymbiotic relationships with insects.

**Obligately anaerobic**
Exclusively anaerobic, i.e. incapable of growing in the presence of free oxygen.

**Parasites**
Parasitic fungi infect and damage plants, animals or other fungi, drawing nutrition from their host.

**Saprotrophs**
Saprotrophic fungi degrade dead organic material, e.g. fallen leaves and twigs, in order to unlock nutrients.

**Spores**
Reproductive structures of lower organisms such as fungi, bacteria, algae, protozoa, ferns and mosses. They may be uni- or multi-cellular and serve as organs of dispersal, survival and sexual or asexual reproduction.

ogy (shape), life cycles and in their dispersal strategies as well as reproduction.

## Where do fungi live?

Fungi grow in almost any habitat as long as organic substrates are available. Even minute nutrient concentrations are sufficient: some fungi may even grow on optical glass and in distilled water. Fungi may also grow on ethanol or gaseous carbon sources such as methane. Members of one group, the Neocallimastigales, show ***obligately anaerobic*** growth in the digestive tract of ruminant animals (cows, sheep) and contribute to their digestive processes.

A pustule of the plant pathogenic rust fungus *Puccinia obscura*, breaking through the epidermis of its host (woodrush) and releasing the spiny urediniospores.

Another kind of symbiosis is that between fungi and ***algae*** or ***cyanobacteria***. The resulting associations (lichens) are typical primary colonisers of extreme habitats such as rock surfaces. Fungi contribute to a large extent to the decomposition of wood in all climatic zones from the antarctic to the rain forest. Wood decomposition releases minerals for renewed use by plants. Soil is also being colonised to a great extent by ***mycorrhizal fungi*** associated with plant roots. The surfaces of plant organs are colonised by fungi which are dispersed through the air. With every breath we inhale numerous ***spores*** of these fungi – much to the distress of asthma sufferers.

The microscopic mould *Wallemia sebi* grows preferentially on sugar-rich substrates, in this case marzipan.

The fly agaric *Amanita muscaria*. This mushroom-type fungus forms mycorrhiza with certain species of trees, especially birch and pines.

Fungi colonise not only terrestrial habitats, but also sea and fresh water. Because of their generally high tolerance of extreme environmental conditions fungi are capable even of colonising substrates with high sugar or salt concentrations, e.g. jams, chocolates, cured sausage and canned fruit.

## How many fungi are there?

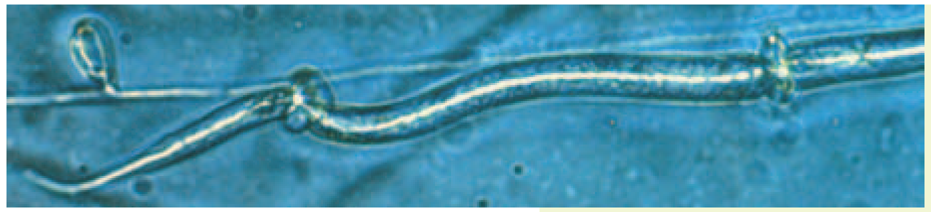
Because of their high ability to adapt to environmental conditions, fungi are ubiquitous and fulfil important roles in all ecosystems. Just as fascinating as the multitude of colours and forms is the enormous potential of producing original and structurally diverse active substances. These may aid in the colonisation of living substrates and serve defence purposes against competitors. Active compounds of fungi have also proven useful for the development of crop protection agents or drugs for medicinal purposes.

At present, some 70,000 to 100,000 species of fungi have been identified and characterised taxonomically. Many more are awaiting their discovery. It is assumed that the total number of fungi may be about 1.5 million. This estimate is based on the following calculation: In Britain approx. 2,000 species of flowering plants are known, and about 12,000 species of fungi have been identified there, additional ones still being discovered. This gives a ratio of at least 6 fungi to one flowering plant. There are about 250,000 species of flowering plants worldwide. Assuming a ratio of 6 fungi to one flowering plant, we obtain a total number of fungi of 1.5 million. Other estimates take into account the immense biodiversity in tropical rainforests, arriving at some 10 million species of fungi. Most experts, however, regard the 1.5 million estimate as realistic. Whatever the total number of fungi, it is apparent that the very large majority of fungal species has not yet been discovered. Fungi therefore represent the largest unexplored biodiversity reservoir on earth. Since the ***secondary metabolism*** of fungi is similarly diverse to that of certain other organisms (e.g. ***Streptomycetes***, ***Myxobacteria*** or sponges), the wide biodiversity of fungi may be expected to yield a similarly large range of natural products. These as yet undiscovered substances represent a huge potential for the development of new products for a multitude of markets, especially in the fields of nutrition, crop protection and pharmeceuticals.

## Fungi – a research area for the future

With only a fraction of fungi identified and cultivated to date, we may expect the discovery of further structurally interesting ***secondary metabolites*** with new biological properties from species not investigated so far. As far as molecular ***genetics*** and targeted ***genome*** mutations are concerned, fungi are much more challenging than bacteria. For this reason, molecular aspects of the ***biosynthesis*** of many important natural products of fungi are as yet incompletely understood.

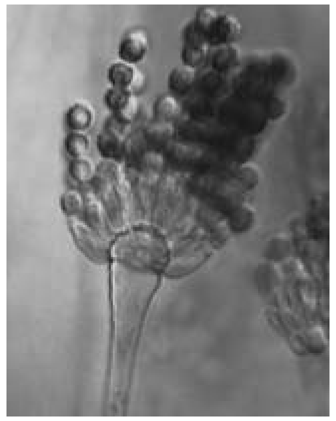
*Beauveria bassiana* is an insect-pathogenic fungus which is sometimes used for biological control purposes.



*Arthrobotrys dactyloides* uses its contractile lassoo cells to trap eel-worms (nematodes).

Therefore, the regulation and recombination of biosynthetic pathways of fungi are promising fields for future research.

Eckhard Thines and Roland W.S. Weber



***Aspergillus penicillioides*** is a mould capable of growing even at very high sugar concentrations or low moisture.

**Molecular genetics**
The science of genetics on the basis of hereditary molecules (DNA and RNA)

**Myxobacteria**
A group of soil-dwelling bacteria which possess an unusually large genome in comparison to other bacteria and are capable of producing a multitude of biologically active secondary metabolites.

**Secondary metabolites**
Products of living cells not required for the basic metabolism of an organism but essential for survival due to their ecological functions (e.g. in defence or flower pigmentation).

**Secondary metabolism**
Synthetic processes whose end products (secondary metabolites) have no direct role in the economy of the living cell. Whereas primary metabolism is more or less conserved in all living organisms, secondary metabolism is often limited to lower life forms and may be species- or even strain-specific.

**Streptomycetes**
Gram-positive bacteria belonging to the Actinomycetes and possessing a highly developed secondary metabolism which enables them to produce a large number of antibiotics of medicinal use as well as other active substances.

**uniquitous**
= to be found everywhere; a ubiquitous organism has a worldwide distribution or may always be found in a given habitat.

*Xanthoria parietina*, a common lichen in our latitudes; branch diameter c. 1.5 cm.

**Biosynthesis**
Synthesis of metabolites from simple precursors mediated by enzymes, e.g. in the production of sugar from carbon dioxide and water.

**Genome**
The total amount of hereditary information within a cell. In bacteria (prokaryonts) the genome usually comprises a circular chromosome and additional plasmids whereas in eukaryotes it is mostly present as a set of linear chromosomes.

## STROBILURINS – FROM NATURAL PRODUCT TO FUNGICIDE

When Timm Anke joined the DFG special research programme "Chemical biology of micro-organisms" at the University of Tübingen in 1975 to initiate his studies on antibiotically active secondary metabolites of basidiomycetes, little previous work had been carried on active compounds in this group of fungi. Mainly some of the medically important toxins such as those produced by the fly agaric or the death cap were known. After initial difficulties in cultivating basidiomycetes had been overcome, these fungi proved to be a good choice. Among the first compounds to be isolated were the selectively antifungal strobilurins from cultures of the European species ***Strobilurus tenacellus***. Molecular structures were elucidated by Wolfgang Steglich's group in Bonn, and the first report on these new compounds was published in 1977. Chemical syntheses and structure-activity studies revealed that the E-β-methoxyacrylate group is essential for the biological activity of these natural products. Neither the free acid nor the Z configuration of this group are active.

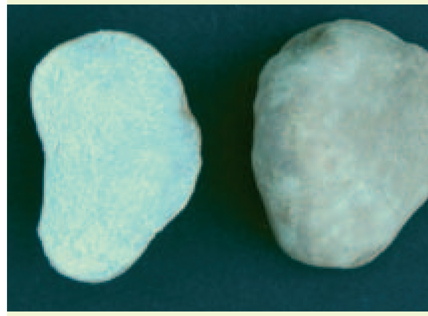
Ergot alkaloids, extracted from the ergots of wheat infected by *Claviceps purpurea*, are used as medication against migraine and for limiting the bleeding after childbirth. These natural products may also be produced by biotechnological fermentations of *Claviceps paspali*.

The mode of action is based on an inhibition of the mitochondrial respiratory chain. Strobilurin-type compounds bind reversibly to the cytochrome bc1 complex, blocking electron flow and the generation of ATP. With the help of strobilurins, G. von Jagow and colleagues were able to elucidate the precise molecular mechanism of electron transfer in this part of the respiratory chain. This respiratory inhibition blocks energy-consuming growth processes such as spore germination of phytopathogenic fungi. In contrast, mammals are able to degrade and thus detoxify strobilurins by the activity of esterases. Some species of ***Penicillium*** also possess such enzymes.

Meanwhile over 20 natural strobilurin derivatives have been isolated from 30 different basidiomycetes worldwide. Most producing fungi are resistant against their own products. In ***S. tenacellus*** this is due to the substitution of three amino acids in cytochrome b. Strobilurins may also be detected in fruit-bodies and the natural environment of fungal producers where they keep competing fungi at bay. Further, strobilurins may be involved in chemical communication between fungi.

The first patent covering the use of strobilurin A as a human antimycotic was submitted in 1980 by the Hoechst company. However, this patent was not pursued further because the antimycotic activity was too weak in comparison with other drugs. It was not until 1983 that that strobilurins were examined for possible use as a crop protection compound by BASF AG in the course of a joint BMBF project. Due to the UV light instability of this natural product, its duration and degree of activity were insufficient for crop protection use. In the following year, a breakthrough was achieved by Wolfgang Steglich and his colleagues who synthesised a much more effective strobilurin derivative. Such enol ether-stilbene derivatives indicated the enormous potential of these fungicides whilst retaining an unacceptably high UV light instability. However, this problem was solved relatively quickly, leading to fierce competition for patent priority between BASF AG and the British ICI company in the following years. The first round with enol ether derivatives was won by ICI, but the equaliser was scored by BASF who submitted their patent application for oximether derivatives two days before their English competitor. Kresoxim-methyl, introduced by BASF in Germany and Belgium in 1996, was the first strobilurin-based crop protection compound. The production facility was located in Brazil. Today fungicides of the strobilurin class belong to the most widely sold crop protection compounds worldwide. Intensive research efforts have resulted in the inclusion of a strobilurin in all major companies' portfolios. Between 1985 and 1997, 400 patents concerning strobilurins have been filed. As early as 1999, these highly effective compounds were employed successfully in wine and fruit production, cereals and special crops in 41 countries. Since 2003, BASF have been marketing their successor product F500 which has successfully saved the Brazilian soybean crop from Asian bean rust. F500 is being produced in a new plant in Schwarzheide, securing employment for 200 staff. The sales volume is estimated to be around 400 million Euro.

In recognition of the successful transfer of strobilurins from university to industry, Timm Anke and Wolfgang Steglich were awarded the Karl-Heinz-Beckurts Prize in 1996.



Fruit bodies of the desert truffle *Terfezia* sp. This fungus enters a mycorrhizal root symbiosis with shrubs growing in extremely hot and dry habitats, securing their survival during periods of drought.

### Additional Literature


Webster J, Weber RWS: Introduction to Fungi (2007), 3. Auflage, Cambridge University Press

Dix NJ, Webster J: Fungal Ecology (1995), Kluwer Academic Press

Müller E, Löffler W: Mykologie (1992), 5. Auflage, Thieme Verlag

Alexopoulos CJ, Mims CW, Blackwell M: Einführung in die Mykologie (1996),
G. Fischer-Verlag


### Links on the Web

Mycology-net
www.mycology.net/index.html

The Fifth Kingdom
www.mycolog.com/fifthtoc.html

The Amazing Kingdom Of Fungi
http://waynesword.palomar.edu/ww0504.htm

Pilze, Pilze, Pilze
www.pilzepilze.de