MASARYK UNIVERSITY, FACULTY OF SCIENCE

DEPARTMENT OF BOTANY AND ZOOLOGY

FUNGAL ECOLOGY

(sometimes with special regard to macromycetes)

Fungi and their environment • Life strategies and interactions of fungi • Ecological groups of fungi, saprotrophs (terrestrial fungi, litter and plant debris, wood substrate, etc.) • Fungal symbioses (ectomycorrhiza, endomycorrhiza, endophytism, lichenism, bacteria, animal relationships) • Parasitism (parasites of animals and fungi, phytopathogenic fungi, types of parasitic relations) • Fungi in various habitats (coniferous forests, broadleaf forests, birch stands and non-forest habitats, fungal communities) • Fungal dispersal and distribution • Threat and protection of fungi

(the study material has not been corrected by native speaker)

LICHENISM

Lichenism is a strong, stable and self-sufficient relationship between fungi (mycobiont) and algae or cyanobacteria (this component is called a phycobiont or cyanobiont, respectively, or generally a photobiont).
The **photobiont** supplies its partner with assimilates, while the **mycobiont** provides a source of water and minerals for the photobiont – so it is not a one-sided „exploitative“ relationship.

In addition, for heteromeric thalli (divided into layers: upper cortical, gonidial with photobiont cells, medullary, or even lower cortical with rhizines on the underside), the mycobiont determines structure of the thallus and provides protection of the photobiont cells against impact of the environment. The upper cortex is of paramount importance as a protection against desiccation, which can be increased by pigmentation of the tissue.

The situation is different in homeomeric thalli (not divided into layers, in the whole volume the cyanobiont cells are scattered among the mycobiont hyphae), where the structure is determined by the cyanobiont, which also secretes polysaccharides capable of absorbing and retaining water (the thallus resembles mucoid colonies of cyanobacteria).

Lichens with cyanobionts are also able to fix nitrogen gas (N_2), while balancing the phosphorus balance, creating growth substances and substances allowing the lichen to grow into the substrate are more provided by the mycobiont.

Reproduction of lichens is mostly vegetative, more rarely mycobiont is forming fruitbodies and spores. In the case of the **fruitbody formation**, the so-called hymenial gonidia can enter the thecuim – photobiont cells, which adhere to the released ascospores.

Different ways of **vegetative reproduction** are the result of an effort to compensate for the low probability of mycobiont spores encounter with the partner's cells – it is a separation of different parts of the thallus (leprose stage) or formation of surface structures such as soredia, isidia, schizidia or phyllidia. /*More about lichen thalli in General mycology, in the chapter Vegetative thallus of fungi.*/

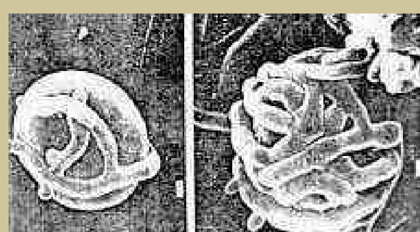
In heteromeric thalli, photobiont reproduction is suppressed (algae or cyanobacteria must be cultivated to detect them), while in the case of homeomeric lichens, the photobiont can grow out from the thallus and reproduce or become independent of the fungus.

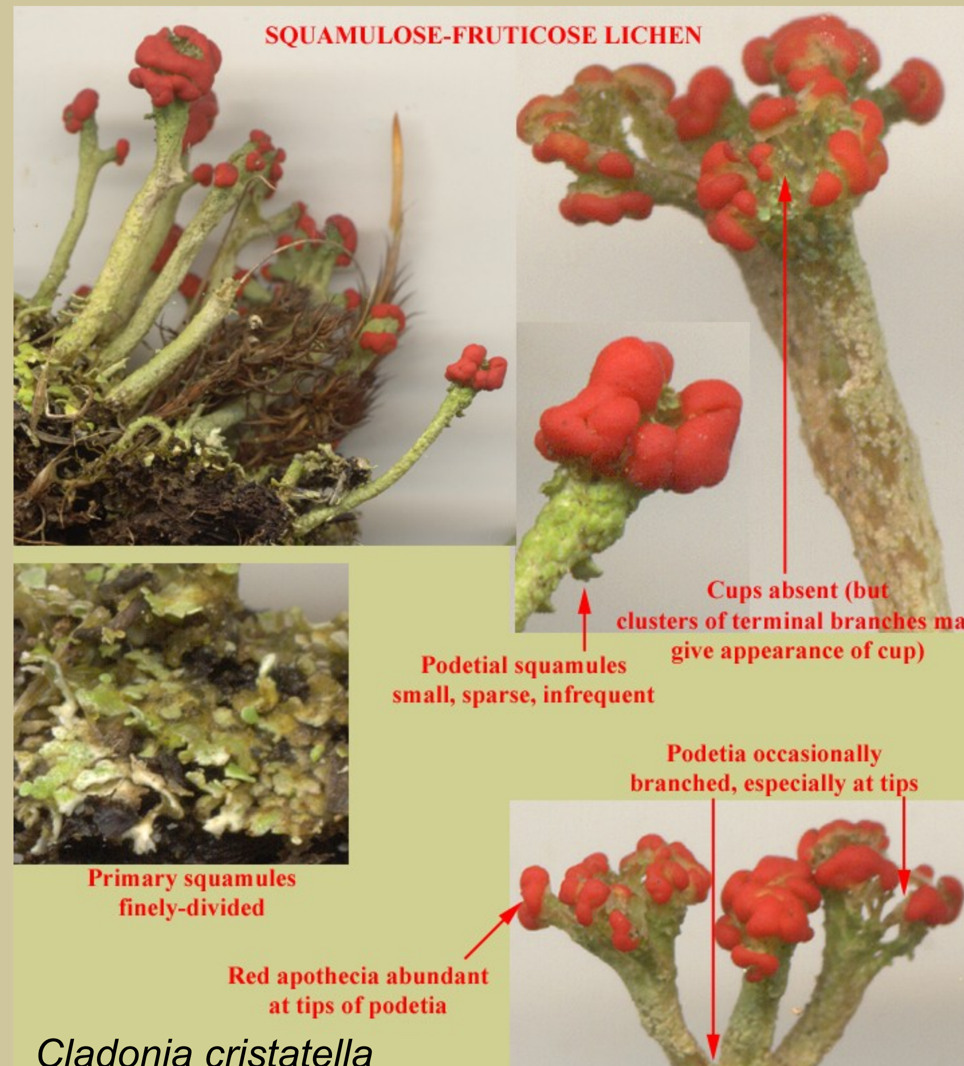
On lichen thalli we can also find fungal parasites (lichenicolous fungi) or parasymbionts (there is no sharp border between these categories). Parasymbiotic fungi are those that live freely (i.e. not in an obligatory relationship), but can enter the lichen symbiosis as an occasional partner (and leave it at any time).

Symbioses are also known at the level of „fellowship“, as well as balanced (mutualistic) and unbalanced symbioses with a tendency to parasitism – many cases of lichenism basically begin as a parasitic relationship, while others gradually turn to parasitism. Cases of lichen „divorce“ have also been observed, what results in (at least temporary) independent existence of its components.

In nature, the behavior of a lichenised fungus was observed on the surface of rocks, where it grows next to colonies of their partner algae or cyanobacteria => fungal hyphae grow to photobiont cells, around which they form mucus and cover them in close contact, and no antibiotic effect was observed – the theory, that lichens evolved as a result of similar interactions, is based on this finding.

Under laboratory conditions, lichen was synthesized only in the second half of the 20th century: mixing a pure culture of fungus (*Cladonia cristatella*) with algal culture (*Trebouxia glomerata*) => hyphae overgrow algal cells => nodules => aggregation => thallus scales formed after about 2 months => fruitbodies formation after some time => complete podetia in about a year.





Cladonia cristatella

In nature, the **lichen growth** is significantly slower, lichens are considered to be the slowest growing organisms – growth of the thallus is usually 0.5–2.5 mm in crustose, 1–6 mm in foliose and fruticose species, at most about 15 mm per year. Age of lichen thalli corresponds to this – they are usually hundreds of years old; crustose lichens, especially in cold regions, can be up to thousands of years old.

Lichens belong to **„S“ strategists** (stress tolerant) with limited competitive ability (this fact is related to slow growth, in addition, lichens do not tolerate shading too much), which can be found in extreme conditions from the tropics (drought in arid regions) to the polar regions (low temperatures).
However, mycobionts of some species also produce antibiotic substances (significantly stronger than common antibiotics), which make them „C“ strategists in principle.

They play an important role in **colonising new habitats** – among their metabolites we find acids that can disrupt the mineral substrate („corrosion“ of the substrate, such as limestone rocks) and release mineral compounds from it for further use by lichens (to a lesser extent; solutions in run-off rainwater or surface water are usually sufficient for them) and for needs of other plant „colonisers“.

Most **lichenised fungi** belong to the division *Ascomycota* (especially the class *Lecanoromycetes*) including imperfect fungi (for which the perfect = ascospore stage is not known); a few percent of the species are then found independently scattered in different groups of the class *Agaricomycetes* (*Basidiomycota*).

A specific case is *Geosiphon pyriforme* (*Glomeromycota*), which contains symbiotic cyanobacteria of the genus *Nostoc* in its thallus (as demonstrated by cultivation). Some lichenologists also classify this organism as lichenised fungus while opponents of this concept argue that it is not a lichen thallus of the heteromeric or homeomeric type – localisation of the symbiont cells corresponds to the definition of endocyanelles as known in some groups of algae (*Glauco-phyta*, some *Dinophyta*). Since all species of *Glomeromycota* have an endomycorrhizal relationship, in this case it may be a combination of two types of symbiosis.

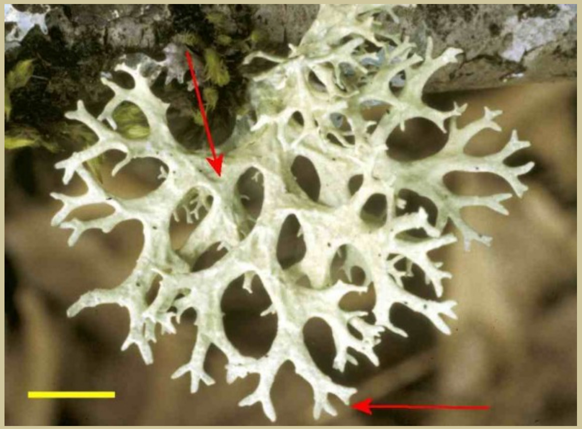
1 *Geosiphon pyriforme*, jeden z nejpodivnějších organizmů, které známe, je blízce příbuzný arbuskulárním mykorhizním houbám. V půdě tvoří nepřehrádkované mycelium, které produkuje kulovité spory a asi 2 mm vysoké měchýřky, obsahující symbiotickou sinici rodu *Nostoc*. Právě tyto měchýřky (znázorněné na obrázku) jsou nejcharakterističtější strukturou tohoto organizmu. Dnes je znám pouze z jediné lokality na světě. Nesnáší zvýšené koncentrace minerálních živin v půdě.

Lichens are known as **indicators of air pollution**; by absorbing practically all substances dissolved in the running water, in their tissues they store compounds which cause dying of the thalli. They are most sensitive to sulphur dioxide, changing chlorophyll to non-green pheophytin => loss of the photosynthetic ability leads to death of the whole organism (externally it is manifested by colour change and gradual disintegration of the thallus).

Such indicators are only some species, it is not possible to apply this role to all lichens – the most sensitive are epiphytic (tree) lichens with fruticose, eventually foliose thallus (*Usnea, Alectoria, Evernia, Lobaria*), ...

Centre: *Alectoria ochroleuca* (Ulrich Kirschbaum, http://kmubserv.tg.fh-giessen.de/pm/page.cfm?PRID=20&CFID=86688&CFTOKEN=154363&PID=901), right: *Evernia prunastri* (Photo Fred M. Rhoades, http://faculty.wwu.edu/fredr/Lichen_table.htm)

Photo Josef Hlásek, http://www.hlasek.com (2x)



... on the contrary, species of the genera *Lecanora* or *Hypogymnia* can be classified as tolerant.

Left: *Lecanora muralis*, right: *Hypogymnia physodes*

Photo Ivo Antušek, http://www.biolib.cz/cz/image/id9453/ http://www.cwikowscy.pl/powiekszenie.php?kategoria=7&nr=1

In nature, there may be places with specific pollution, for example with a high concentration of some metals, where species tolerant to these metals can selectively succeed; on the contrary, in cities the pollution is usually „complex“, so the spectrum of lichen flora is significantly limited.

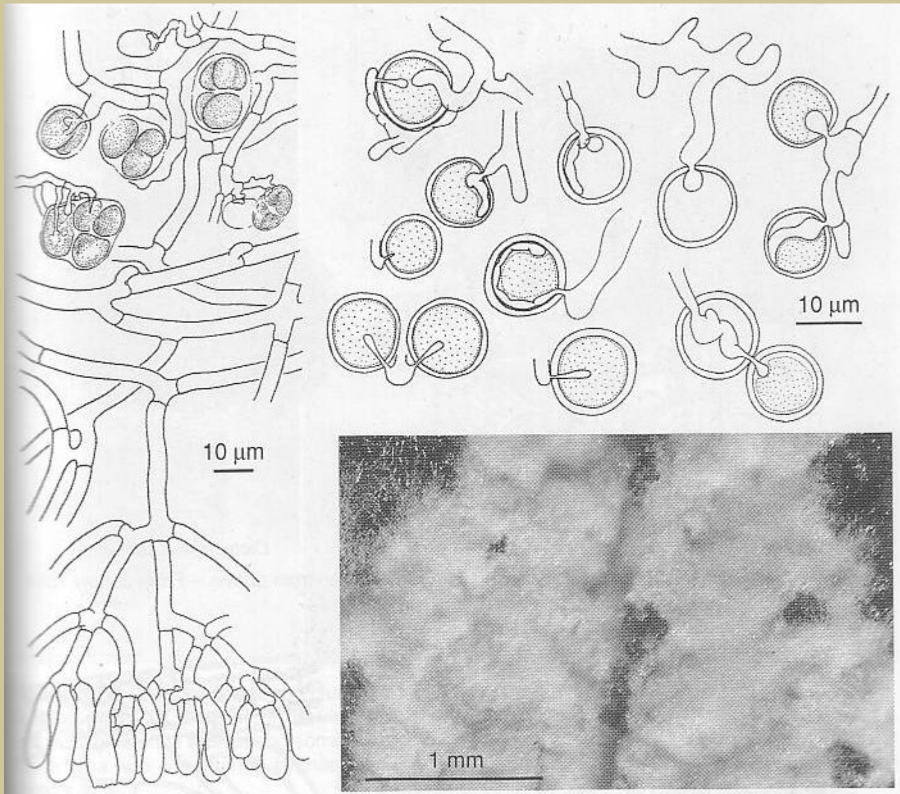


Figure 11.6: *Athelia epiphylla* is a parasite on unicellular green algae living on wood, bark or dead leaves, or present in the thallus of the lichen *Lecanora*. The algal cells are penetrated by haustoria and killed. – Basidiome section from Oberwinkler 1970, algal cells with haustoria from Poelt & Jülich 1969. Basidiome: original photograph.

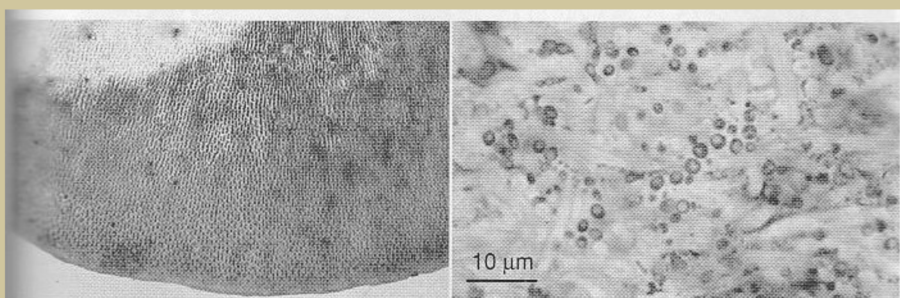


Figure 11.4: The basidiomes of *Trametes gibbosa* are frequently colonised by various green algae that may penetrate deep into the context. **Left:** The dark zone of the hymenophore is of a saturated green colour caused by the algae. **Right:** Section through the basidiome context showing unicellular green algae and thick-walled fibre hyphae. – Original photographs.

Clémençon: Cytology and Plectology of the Hymenomycetes, 2004.

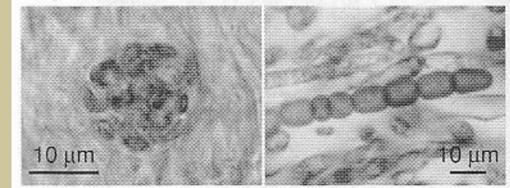


Figure11.3: Accidental associations: **Left:** A colony of green algae in the subhymenium of *Peniophora piceae.* Original photograph. **Right:** A trichome of *Anabaena* in the gill trama of *Crepidotus mollis.* From Clémençon 1997.

In addition to lichenism, there are also **looser interactions** between different species of **fungi and algae** – for example, different *Trametes* species, whose fruitbodies tend to be colonised by algal cells over time (not only on the surface, sometimes entering deep into the tissues), or corticioid fungi growing on the surface of wood previously colonised by algae; species of the genus *Athelia* thus pass to direct parasitism, the penetration of haustoria and killing of algal cells – not only of free algae, but also of lichen photobionts (observed on *Lecanora conizaeoides*).

The mentioned cases are usually „accidental and irregular encounters“, but there are also frequent coexistences such as *Resinicium bicolor* with chlorophyte *Coccomyxa glaronensis* – although it is not obligatory lichenism, fungal structures or algal cells are not morphologically adapted, the algal cell involvement in the fruitbody tissue is referred to as „physiological lichenism“.

Heinz Clémençon: Cytology and Plectology of the Hymenomycetes. Bibliotheca Mycologica 199. J. Cramer, Berlin-Stuttgart, 2004.

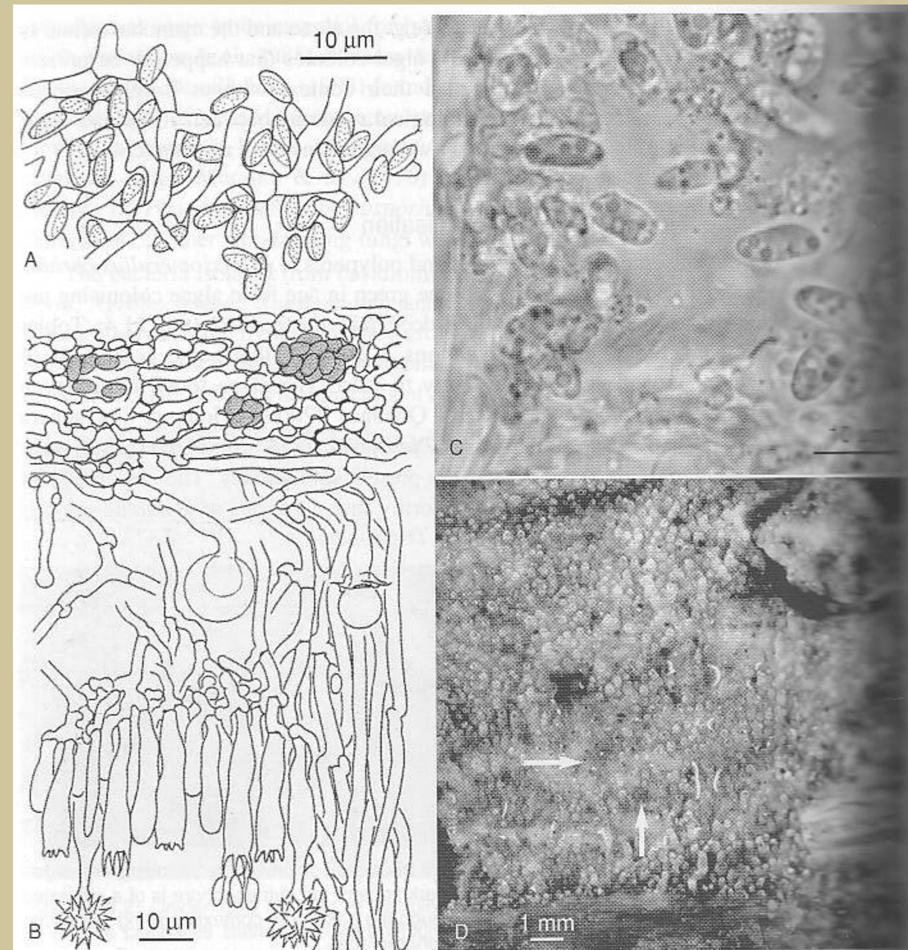


Figure 11.5: *Resinicium bicolor* is usually associated with unicellular green algae of the [illegible] *Coccomyxa* without forming any structure to accommodate or to exploit the algae. **A:** Hyphae [illegible] algae, from Poelt & Jülich 1969. **B:** Section through a basidiome, algae grayed. From [illegible] 1970, modified. **C:** *Coccomyxa* cells in a squash mount of a basidiome. **D:** Mature basidiome. [illegible] algal mats appear as darker areas (two indicated by **arrows**). – Original photographs.

INTERACTIONS WITH BACTERIA

Cyanobacteria in lichens are not the only prokaryotes in which a relationship with fungi has been observed – during the 20th century, **coexistence with bacteria** was discovered (first in some gasteromycetes, later also in other groups of fungi). It can be assumed that mutual interactions (as well as competition for resources) have been here since living organisms became terrestrial.

Bacteria are abundant in the rhizosphere, on the hyphal surface, but also inside the hyphae. They are also present in mucoid colonies inside the tissues (in the space between the hyphae), or in the mucus layer on the surface of the fruitbodies (ixocutis); in larger quantities they occur, for example, in the fruitbodies of *Hydnum rufescens* or *Cantharellus cibarius*, where they seem to „help“ in the fruitbody development.

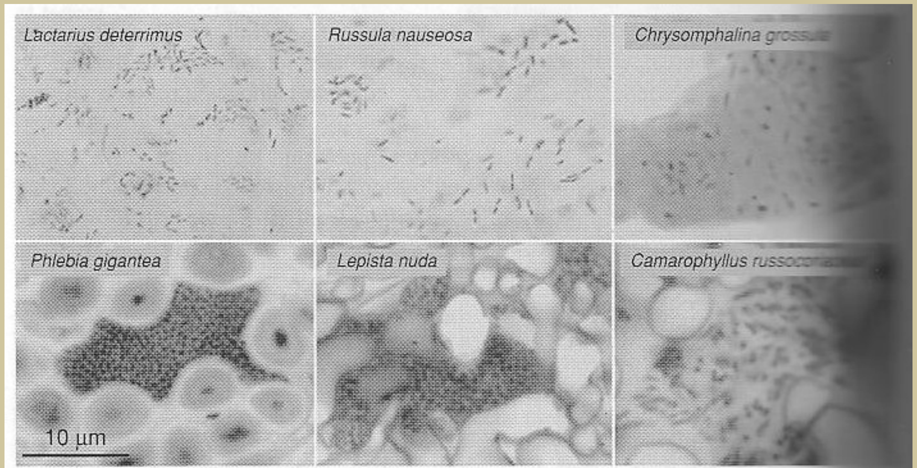
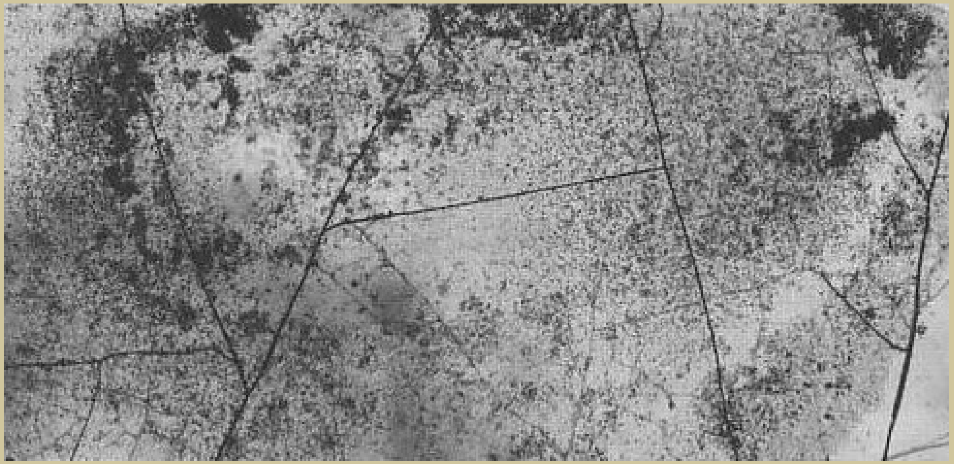


Figure 11.2: Unidentified bacteria in basidiomes. **Top:** *Lactarius, Russula:* Acid-fast ... gelatinous pileipelles stained with carbol-fuchsin and mounted in lactic acid - glycerol. ... *phalina*: Bacterial colony in gill trama, basic fuchsin. **Bottom:** *Phlebia:* Bacterial ... subiculum, ruthenium red; *Lepista:* Bacterial colony in the nodulus, haematoxylin; ... Bacterial colony in the gill trama, basic fuchsin. – Original photographs.

Heinz Clémençon: Cytology and Plectology of the Hymenomycetes. Bibliotheca Mycologica 199. J. Cramer, Berlin-Stuttgart, 2004.

Together with fungi, bacteria are involved in the degradation of root exudates (the original ideas even attributed this role exclusively to bacteria). As ^{13}C has shown, fungi are most involved in the degradation of exudates in acidic soils and at high concentrations of exudates (they better tolerate osmotic stress), otherwise competition (associated with excretion of anti-fungal, anti-bacterial and non-specific substances) is to be expected.



„Dirt“ among dark hyphae of the genus *Rhizoctonia* (see also a more detailed picture of monilioid cells in orchid mycorrhiza) are ubiquitous bacteria.

Gryndler et al.: Mykorhizní symbióza, Academia, Praha, 2004.

Some bacteria manifest as mycophagous. Spore penetration and wrapping of *Streptomyces* chains around the hyphae were observed; species of this genus are also the main decomposers of dead hyphae in soil. The genus *Collimonas* has recently been found to attack fungal hyphae in acidic soils and to penetrate their walls with a mixture of enzymes (chitinases, proteases). A specific case is myxobacteria (G–), which commonly feed on other bacteria and yeasts („micropredators“), but their motile mucoid colonies have been seen attacking the hyphae of *Rhizoctonia* sp.

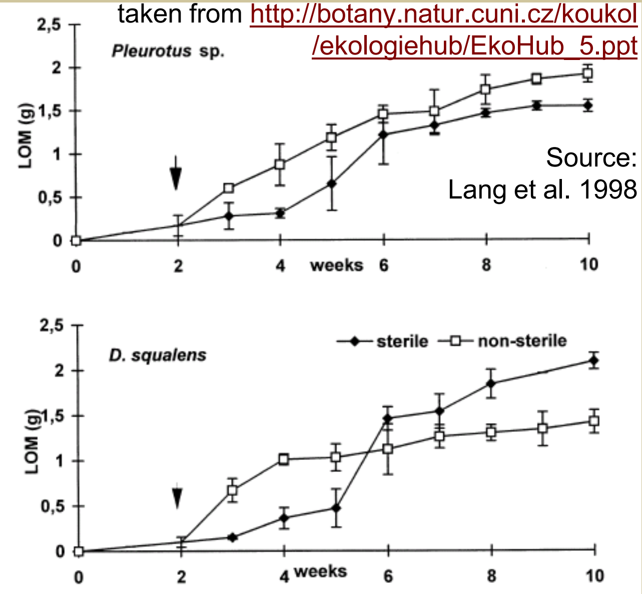
Some bacteria are mycoparasitic (e.g. *Pseudomonas tolaasii* on *Agaricus bisporus*).

During decomposition of lignocellulosic complex, simple sugars and phenolic compounds are formed – the interactions that occur here can be mutualistic, commensal or even competitive:

– bacteria utilise nutrients without fungus suffering, while they are producing vitamins, growth factors, fixing nitrogen, breaking down toxic metabolites;
– bacteria utilise nutrients without fungus suffering;
– bacteria utilise nutrients and „rob“ the fungus.

Growth of bacteria and actinomycetes on lignin has also been observed in vitro, but under natural conditions their proportion is negligible and lignin degradation is a matter of fungi. Also aerobic decomposition of cellulose is mainly a matter of fungi, but in anaerobic conditions (with the exception of *Neocallimastigales*, see below) bacteria are involved (*Acetivibrio, Clostridium*). Aerobic bacteria and actinomycetes need higher pH than fungi – they succeed e.g. in composts, where the pH increases by ammonification (but there is also competition of fungi and bacteria), while in soil they occur only opportunistically (they probably do not have sufficient antifungal substances) and in wood minimally (it is an acidic substrate itself, into which the fungi also excrete organic acids).

taken from http://botany.natur.cuni.cz/koukol/ekologiehub/EkoHub_5.ppt



Source: Lang et al. 1998

Fig. 1. Loss of organic matter of the straw compartment during growth of *Pleurotus* sp. strain Bhutan and *Dichomitus squalens* in solid state straw-soil cultures in the presence (nonsterile soil, open symbols) and absence (sterile soil, solid symbols) of soil microorganisms. Arrow indicates the time soil was added. Mean values and standard deviations of four replicates are given.

Different positive/negative affect of bacteria on growth of different fungi

A specific niche for bacteria is the surface of hyphae, where they utilise mannitol and trehalose (*Pseudomonas*) or organic acids (*Methylobacterium, Streptomyces*).

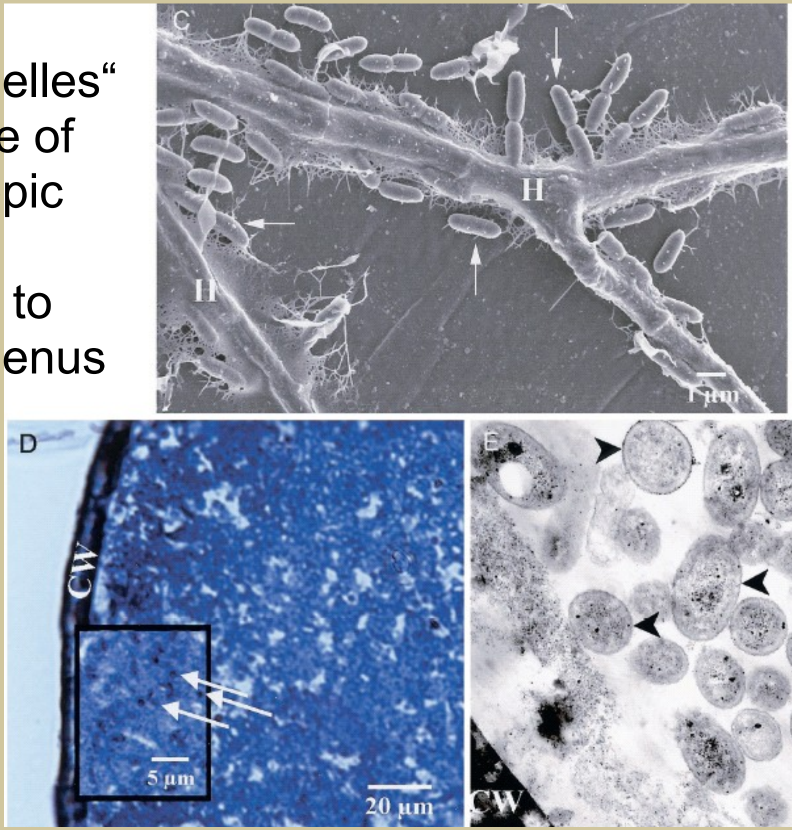
The production of fungal substances has not yet been quantified here, but apparently the fungi affect the qualitative spectrum more than the quantity of bacteria, while the selection of bacteria resistant to fungal antibiotics (G+ versus G–) is also evident.

Source: R. Campbell: Plant Microbiology. Edward Arnold, London, 1985
http://soils.usda.gov/sqi/concepts/soil_biology/bacteria.html
(published there with permission of Cambridge University Press)

Different communities can be found in the rhizosphere without mycorrhiza and in mycorrhizosphere due to different production of fungal exudates and stimulation of the plant exudation. Group of species mainly of the genus *Pseudomonas* represents the so-called „ECM helper bacteria“ – they stimulate the formation of mycorrhizal symbiosis and the growth of ectomycorrhizal fungi (the mechanism of action remains unknown). Some of themalso have an effect on fructification (*Agaricus bisporus*, *Pleurotus ostreatus* + *Pseudomonas putida*), in other cases the fungus benefits from the interaction with the plant and nitrogen-fixing bacteria (community with mycorrhiza of *Rhizopogon vinicolor* + *Pseudotsuga*).

Arbuscular fungi do not have association with true „helpers“, but there is positive effect of fungi and bacteria on the plant, the spread of bacteria on the mycelium (*Rhizobium leguminosarum* + *Gigaspora margarita*) or the germination of spores of mycorrhizal fungi if antifungal substances are degraded (on the other hand, their production has a negative effect on the spore germination => induction of mycostasis or fungistasis).

Since the 1970s, „bacteria-like organelles“ had been observed inside the hyphae of arbuscular fungi in electron microscopic images, in which bacteria were recognised (non-cultivable, identified to species by PCR), most often of the genus *Burkholderia* (the original idea, that they help with nitrogen fixation, has not been confirmed). Probably a symbiotic event happened only once, followed by vertical transmission.



Source: Levy et al. 2003; taken from http://botany.natur.cuni.cz/koukol/ekologiehub/EkoHub_5.ppt

C: SEM photo of *Gigaspora decipiens* hyphae (H), *Burkholderia pseudomallei* (arrows) and fibrillar material. **D:** Semi-thin section of *G. decipiens* hypha inoculated by *B. vietnamiensis*; stained by toluidine blue. Bacteria (arrows) are present everywhere in the cytoplasm (CW = cell wall). **E:** TEM photo of bacteria in the cytoplasm of *G. decipiens* (arrows).

The transfer of bacterial cells appears to take place through the spores, and entry into the mycelium can occur when the wall of the germ tube is disrupted by lytic enzymes. Otherwise, the „absorption“ of bacteria is difficult due to strength of the cell wall and it can occur only at the tip during hyphal growth (this mechanism also has *Geosiphon pyriforme* + *Nostoc*; there is something like phagocytosis and formation of vesicles at the tips of the hyphae).

By the way, bacteria of the genus *Burkholderia* are common colonisers of the rhizosphere that can utilise almost anything (they even degrade halogen derivatives). Their coexistence with other organisms can take various forms (beneficial to plants or parasitic) and they can attack eukaryotic cells and survive as intracellular parasites (observed in amoebae, but also in macrophages, epithelial cells); pathogenic effects on animals, including humans, are also possible (e.g. *B. cepacia* and *B. pseudomallei* can kill people with weakened immunity, after application of antibiotics or e.g. with cystic fibrosis).

Burkholderia cepacia and symptoms of its action in onions