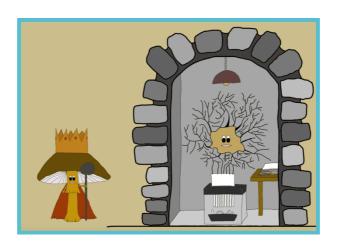
# Single-sided



ACCOMPANYING MATERIAL FOR THE EXPERIMENT WEBSITE WWW.KNIFFELIX.DE ON THE SUBJECT OF

# The underestimated kindom: The fungus and its mycelium



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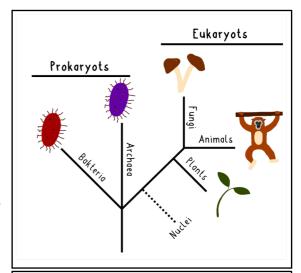


#### Knowledge box I: Evolution and classification of fungi

Fungi come in many colors and shapes - they grow in water or on land and can be useful or harmful. But have you ever wondered what fungi actually are? For a long time, they were classified as plants, but today we know that they form their very own kingdom. But what exactly does that mean? And how do they differ from plants and animals?

#### What is biological classification?

There are millions of different species in the world, so it can be difficult to keep track of them all. Imagine if all these species were products in a supermarket, but everything was randomly placed on the shelves. To make it easier to find food, it is sorted into categories such as fruit, vegetables, or dairy products. Biological classification works in the same way: living organisms are divided into groups based on their similarities

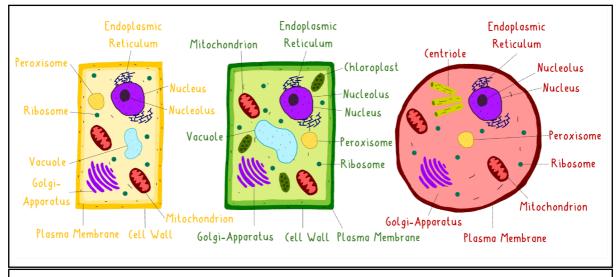


Picture 1: Simplified family tree of life. The branches show how different groups of living organisms developed from common ancestors.

(Picture 1). At the very beginning, a distinction is made between organisms with a cell nucleus (eukaryotes) and those without (prokaryotes). Eukaryotes are further divided into the life forms plants, animals, and fungi. Until the late 20th century, it was thought that fungi belonged to the plant kingdom. Today we know that they form their own group and are even more closely related to animals than to plants.

#### What distinguishes animals, plants and fungi?

Most animals can be distinguished from plants or fungi at first glance because they can move. But how is it possible that fungi, which look so similar to plants, are actually more closely related to animals? To understand this, we need to take a closer look at cells.



Picture 2: Drawing of the cells of fungi (yellow), plants (green), and animals (red) with their most important components.





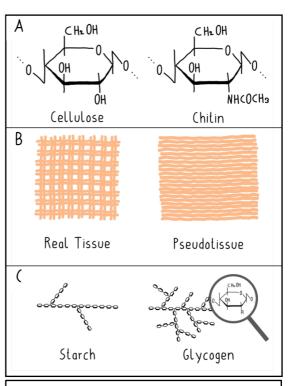






#### A look inside the cell

All living organisms are composed of cells surrounded by a protective membrane. These cells contain ribosomes, which link amino acids to form protein chains (Picture 2). Eukaryotes—animals, plants, and fungi-also have a cell nucleus that contains their genetic material, known as DNA. If you take a closer look at the cell structures of animals, plants, and fungi, you will see many similarities: they have mitochondria for energy production, an endoplasmic reticulum (ER) in which a large number of proteins are produced, a Golgi apparatus responsible for the modification, sorting, and transport of proteins, and peroxisomes for the breakdown of harmful substances. But there are also crucial differences. Plant cells have a cell wall made of cellulose, which gives them stability. They also have chloroplasts, which they use to convert sunlight into energy, and a large vacuole for storing fluids. Animal cells, on the other hand, have neither a cell wall, nor chloroplasts, nor a vacuole, but they do have centrioles, which play a role in cell division. Fungal cells are, in a sense, a mixture of both: they have a cell wall and a vacuole like plant cells, but their cell wall is not made of cellulose, but of chitin – a substance that is



Picture 3: Important characteristics for comparing plants, animals, and fungi: A. Components of the cell wall, B. Tissue types, C. Storage form of sugar.

also found in insect shells (Picture 3A). They also lack chloroplasts, which is why, unlike plants, they do not obtain energy from sunlight.

Table 1: Similarities and differences between animals, plants, and fungi.				
	Topic	Fungus	Plant	Animal
(ellular differences	(ell Wall	Chitin	(ellulose	Not Present
	Vacuole	Present	Present	Not Present
	(hloroplasts	Not Present	Present	Not Present
Differences whole organism	Tissue	Pseudotissue	Real tissue	Real Tissue
	Nutrition	Decomposing	Photosynthesis	Decomposing
	Sugar Storage	Glycogen	Starch	Glycogen
	Mobility	Not Present	Not Present	Present



## **Fungi**







#### Other differences

Beyond cell structures, there are also major differences between plants, animals, and fungi. While plants and animals have real tissue in which cells are connected to each other, fungi consist of cell filaments that are only loosely connected to each other, a so-called pseudotissue (Picture 3B). The way they obtain food also differs: plants can produce sugar themselves through photosynthesis and store it as starch. Animals and fungi, on the other hand, must obtain sugar from the environment, but store it in the form of glycogen, which is more branched than starch (Picture 3C). Another significant difference can be seen in digestion. Animals ingest food through their mouths and break it down in their bodies with the help of enzymes. Fungi, on the other hand, excrete their enzymes externally, which means that their food is already broken down outside the body before the nutrients are absorbed. Table 1 provides an overview of the similarities and differences between animals, plants, and fungi.

The fact that fungi are more closely related to animals than to plants was not only determined on the basis of these characteristics, but was also confirmed by DNA analyses. A comparison of the genetic material of fungi with that of plants and animals shows that fungi are indeed genetically more similar to animals. These findings have led to fungi now being considered a separate group within the living world and no longer being classified as plants.









#### Knowledge box II: The diversity of fungi

With an estimated 3.8 million species, the kingdom of fungi is more than six times larger than the kingdom of plants. But how can you tell all these species apart?

#### How can mushrooms be categorized?

In the past, mushrooms were identified by sight, smell, and touch. Attention was paid to macroscopic characteristics such as color, shape, and odor. It was assumed that species that looked similar were also closely related. Over time, new methods of mushroom identification were developed. Microscopy helped to reveal finer details that are not visible to the naked eye. This made it possible to discover even more species and distinguish them based on their appearance. The biggest change in mushroom research came with DNA analysis. It allows researchers to examine the genetic material of mushrooms and thus find out how Closely related different species actually are. This revealed some surprising findings: Some mushrooms that look confusingly similar on the

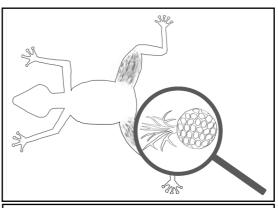
outside are not as genetically related as previously thought. Despite these advances, however, it is still difficult to classify fungi accurately according to their relationship, mainly because many species remain undiscovered to this day. This leaves large gaps in our knowledge of how fungi have evolved over time. When classifying fungi, it is therefore still common to use form classes—that is, external characteristics such as appearance and shape. This is particularly useful when collecting fungi in the forest, because it is less about genetic relatedness and more about the question: edible or poisonous?

Picture 4: Microsporidia can cause eye diseases in humans.

#### Which types of fungi are known?

Based on DNA analyses, six groups can be distinguished.

- 1. **Microsporidia** (Picture 4): These are the smallest fungi—and even the smallest known organisms with a cell nucleus! They consist of only one cell and live as parasites in animals, sometimes also in humans. In healthy people, they cause at most mild diarrhea or eye infections. However, they can have dangerous effects on people with weakened immune systems, such as HIV patients.
- 2. **Chytrids** (Picture 5): These fungi usually live in water. They are often single-celled and parasitic. Their spores have small flagella, i.e. filamentous appendages, which they use to move around in the water.

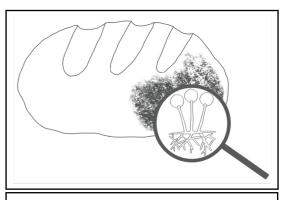


Picture 5: The chytrid fungus attacks the skin of amphibians and often leads to fatal infections, causing entire species in Australia and South America to become extinct.

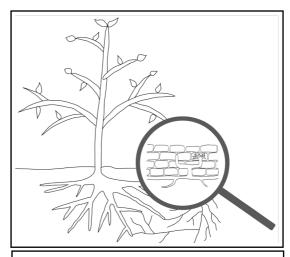




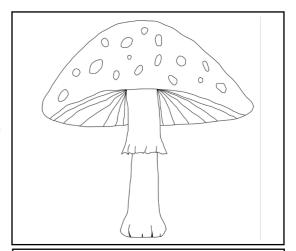
- 3. **Zygomycota** (Picture 6): They live almost exclusively on land and can form a network of filamentous cells called mycelium. Unlike other fungal groups, these filaments are not separated by cell walls. Many molds, such as bread mold, belong to this group.
- 4. Glomeromycota (Picture 7): Mycorrhizal fungi live in close symbiosis with plants: they supply water and nutrients from the soil and receive sugar from the plant in return. Arbuscular mycorrhizal fungi—the largest group of these symbiotic fungi—form particularly close connections. Their fine fungal threads penetrate directly into the roots of the plants to supply them even better. Since they grow underground and do not form visible fruiting bodies, they remain invisible to us.
- 5. **Basidiomycota** (Picture 8): These fungi are particularly well known because they form the typical fruiting bodies in the forest from cap mushrooms to spherical puffballs and tree fungi. Many basidiomycetes live in mycorrhizal symbiosis with trees. Other species infest plants as parasites, decompose dead wood, or even grow on other fungi. Some yeast fungi also belong to the basidiomycetes, but play a less obvious role in everyday life than the yeast fungi of the following group.
- 6. Ascomycota (Picture 9): This group of fungi is extremely diverse and we encounter it often in everyday life. Many yeasts belong to this group, including the well-known baker's yeast, which is used in the production of bread, beer, and wine. Some intestinal fungi also belong to the yeast fungi. Alongside the zygomycetes, the ascomycetes are one of the two groups of fungi from which **molds** have developed. Some cause food such as fruit to spoil, while others are useful, such as the Penicillium fungus, from which antibiotics are obtained. More rarely, there are also mycorrhizal fungi among them, which live in association with plants. These include truffles and morels, which form underground or above-ground fruiting bodies and are prized as delicacies.



Picture 6: Bread mold is a fungus belonging to the group of zygomycetes.



Picture 7: An arbuscular mycorrhizal fungus grows into the roots of the plant, right into the cells, and helps it absorb nutrients from the soil.

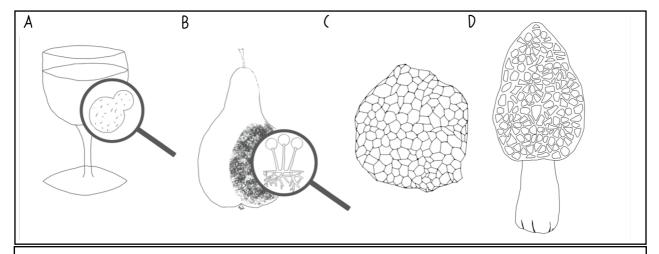


Picture 8: The visible fruiting body of a stalked fungus.









Picture 9: Different types of fungi. A: Yeast fungi ensure that wine contains alcohol. B: Mold fungi decompose old fruit. C: Truffle fungi form their fruiting bodies underground. D: Morels form distinctive fruiting bodies above ground.

Although this classification is based on genetic analyses, it is not definitive. Research into the genetic diversity of fungi is still in its infancy, and future findings could further change the classification. Nevertheless, this classification helps to understand the wide range of fungal species and their habitats. Molds in particular show that similar fungi can have very different effects—some are useful, others harmful. But what determines whether a fungus is a friend or foe?

#### What makes a fungus a foe?

Fungi play an important role in nature because they break down old materials and release nutrients. This ability is often useful – but not always desirable. For example, a fungus that breaks down wood can become a problem when it spreads in buildings and destroys the building fabric. Even more worrying is the idea that some fungi can even attack and break down animal tissue or organs. Fungi can also cause imbalances in the human body. Yeast fungi are a natural part of the intestinal flora, but if they multiply uncontrollably, they can inhibit the growth of important bacteria that also live in the intestine and disrupt the balance in the intestine. In addition, there are fungi that produce toxic substances – known as mycotoxins – which can cause illness or allergic reactions. Despite these risks, most fungi are not harmful to humans or nature. On the contrary – they are essential for a healthy ecosystem!

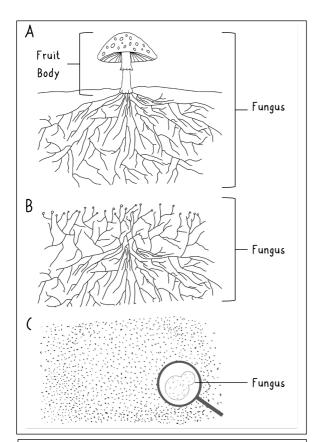








#### Knowledge box III: Life cycle



Picture 10: Different forms in which fungi can grow. A: Fungi with mycelium and fruiting bodies B: Fungi with mycelium without fruiting bodies C: Single-celled fungi without mycelium.

hyphae gradually spread out and form a fine network – the socalled **mycelium**. The fungus needs nutrients to grow. Since it cannot move, it simply grows towards its food source. Unlike plants, it does not need light to do this. That is why it usually grows in the soil – and often remains invisible to us.

However, when it comes to fungi, a distinction must be made from the outset between single-celled and multicellular organisms.

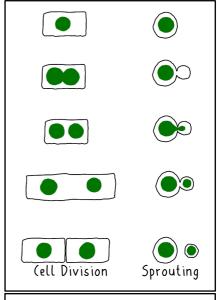
Multicellular fungi (Picture 10A,B) form a mycelium from the fungal threads already described, the hyphae, but not necessarily a fruiting body. Above-ground growth serves to spread spores and absorb oxygen. For this reason, we can marvel at more complex structures such as the familiar cap mushrooms in the forest, especially in autumn. Other multicellular fungi remain permanently in thread-like form and develop spore-forming structures that are invisible to the human eye.

#### What is a fungus?

When we think of fungi, most of us imagine a mushroom with a cap sprouting from the ground in autumn. But what we see is only a small part of certain types of fungi—the so-called fruiting body. Many fungi, however, do not form a fruiting body at all. The actual fungus usually grows invisibly underground as a fine network of filaments called mycelium. But how does a fungus generally develop—and how does it grow?

#### How does a fungus grow?

Most fungi develop from **spores**, which, similar to plant seeds, serve to spread and reproduce the fungus. However, while plant seeds are much larger and consist of an embryo and nutrient tissue, spores are usually tiny and single-celled. They are extremely resilient and can survive for a long time – even in extreme heat, cold, or dry conditions. This property ensures that the fungus has a greater chance of survival in extreme weather conditions. In order for the fungus to colonize new areas, spores are transported for example by wind, water, animals, or humans. When the spore encounters favorable conditions, it germinates and forms fungal threads. The fine threads of a fungus are called **hyphae**. These



Picture 11: This is how fungi reproduce: through cell division and budding.



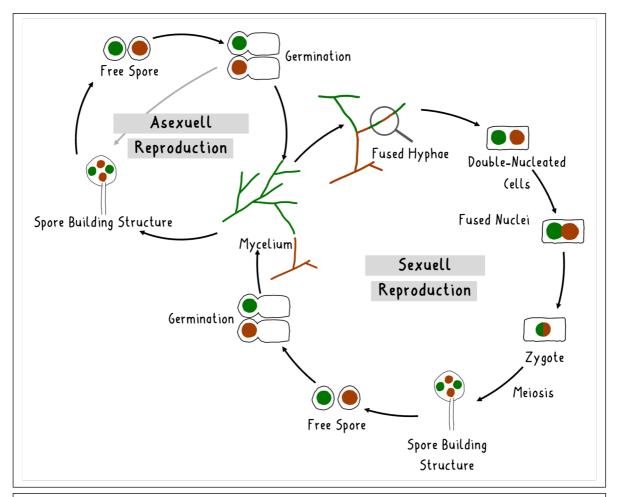




- **Single-celled fungi** (Picture 10C), on the other hand, do not form mycelium and reproduce mainly by dividing individual cells. A well-known example are yeast fungi, which are used in the production of bread, wine, and alcoholic beverages. A special feature of yeast cells is their reproduction by budding or sprouting. In contrast to classic cell division, in which two daughter cells of equal size are produced, sprouting involves the formation of a small protrusion on the mother cell, from which a smaller daughter cell initially develops (Picture 11). Yeast fungi form spores under certain conditions – for example, at optimal temperature and humidity or under stress such as nutrient deficiency. This increases their chances of survival and reproduction under changing environmental conditions.

#### How do fungi reproduce?

Fungi can reproduce both asexually and sexually (Picture 12). In asexual reproduction, spores are formed that grow into new fungi without the need for a second fungus or any change in genetic information. Sexual reproduction works differently: fungi do not have just two sexes like humans, but many different sexes with different mating types. They cannot be distinguished externally – they can only be identified through laboratory analysis. When two hyphae of different mating types meet, they can fuse together and contain two cell nuclei that can coexist for a certain period of time. Depending on the species, this state can last for varying lengths of time – only briefly in some fungi,



Picture 12: The life cycle of a fungus – from spore to mycelium to new spore.













but for years in others. At some point, these cell nuclei fuse and exchange genetic material. This creates new spore-forming structures that start the reproductive cycle all over again.

#### When does a fruit body form?

The visible fruiting body is only part of the life cycle in some species. In fruiting body-forming species, however, it develops when two hyphae of different mating types fuse (Picture 13). It can then grow out of the ground and become visible to us. Inside the cap, new spores are formed in the gills or tubes, for example. The fruiting body of a fungus can be compared to the fruit of a plant. Its main purpose is to enable reproduction by spreading spores. While the cap usually only forms at certain times of the year, the mycelium remains in the soil throughout the year and can grow to enormous proportions. The largest known mycelium is an impressive 9 km2 in size – that's about 1,260 soccer fields! Most of a fungus is therefore invisible to us, as it spreads underground or in wood, for example. What we colloquially perceive as a fungus is often only the "tip of the iceberg."









#### **Knowledge box IV: Fungal nutrition and its benefits**

#### How do fungi feed?

Fungi are fascinating organisms with a unique way of feeding. Since they cannot move, they have to literally grow through their food in order to access new food sources (Picture 14). Different types of fungi prefer different organic materials.

Many fungi feed on dead plants and animals and play an important role as decomposers in nature. They help break down dead material and return nutrients to the natural cycle. This group of fungi also includes molds, which we often encounter in everyday life as they grow on (old) food.

However, there are also fungi that feed on living organisms. Some form a symbiotic partnership with plants, such as mycorrhizal fungi. They help plants absorb water and nutrients from the soil,

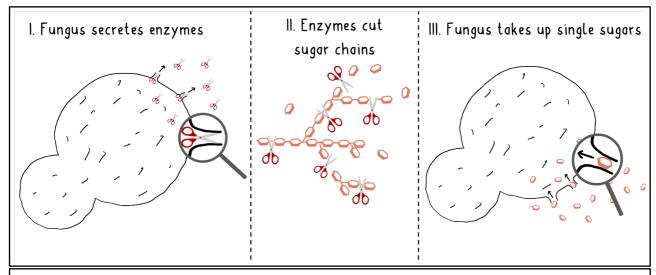


Picture 14: Fungus grows through strawberries and decomposes them, making them mushy.

while in return they receive sugar from the plants. Around 90% of all plants are connected to fungi via their roots. There is evidence that this symbiosis not only supports plant growth, but may also play a role in communication between trees. However, the relationship between fungi and other organisms is not always harmonious. Some fungi act aggressively and attack insects, for example, growing through their bodies and ultimately killing them.

#### How do fungi absorb nutrients?

In order to absorb nutrients, fungi must digest them outside their bodies. While humans break down their food by chewing and then process it further with enzymes in the stomach, fungi release enzymes into their environment (Picture 15). These enzymes break down large molecules into smaller ones, which the fungus can then absorb. Fungi are highly specialized in this process and,



Picture 15: A yeast fungus breaks down long-chain sugars and extracts energy from them. Scissors: enzymes, hexagons: sugar molecules.





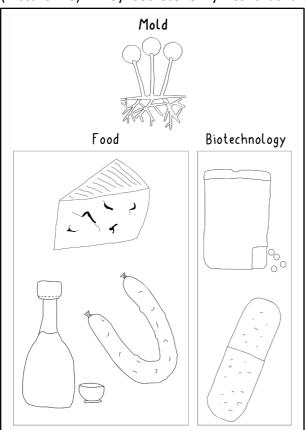


depending on the species, only secrete certain enzymes to break down specific materials. They can break down sugars, proteins, fats, and even the hard lignin found in trees. This specialization shows how important a diverse mix of fungi is for the ecosystem, as together they can break down a wide range of organic material. However, this ability is not only important for nature, but also for various production processes in different branches of industry. Yeast and molds play a major role here.

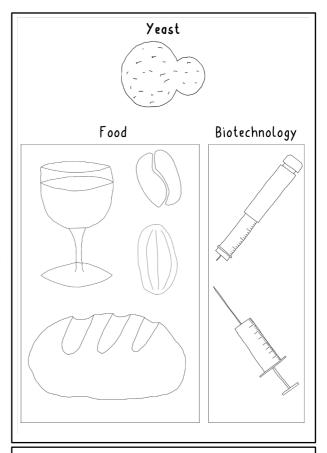
# How do humans use the decomposition ability of fungi?

Fungi play an important role in industry because they break down substances and produce valuable by-products in the process. This process is known in industry as fermentation.

The use of **yeast fungi** is particularly well known (Picture 16). They secrete enzymes that break



Picture 17: This is how molds are used in industry – for example, in food production and biotechnologically manufactured medicines.



Picture 16: This is how yeast fungi are used in industry – for example, in food production and biotechnologically manufactured medicines.

down starch. Starch consists of long chains of sugar molecules. The enzymes secreted by yeast break down these long chains (starch) into individual sugar molecules. These are absorbed by the yeast and further broken down to produce energy. This process produces ethanol (alcohol) and carbon dioxide (CO<sub>2</sub>) as by-products of sugar breakdown. This process is called alcoholic fermentation and is mainly used in the production of beer and wine. The carbon dioxide makes the drinks sparkling. Yeast also plays an important role in baking: during baking, the carbon dioxide produced loosens the dough, making bread and rolls nice and airy. The alcohol simply evaporates in the oven. Less well known, but equally fascinating, is the role of yeast in the processing of cocoa and coffee beans. They ensure that the sweet pulp of the beans is broken down – a crucial step that gives the beans their distinctive aroma and chocolate and coffee their characteristic taste.

## **Fungi**







In addition to food, yeast fungi are also a great help in biotechnology: through targeted genetic modifications, they can be made to produce and excrete important substances such as **insulin or vaccines.** 

Another important group of fungi in industry are **molds** (Picture 17). Unlike yeasts, they can break down not only sugars but also fats and proteins. They are often used to produce certain flavors, for example in **blue cheese or soy sauce**. A white mold layer is also deliberately formed on certain **types of salami**. This layer breaks down fats and proteins into smaller components, intensifying the flavor. At the same time, it protects the salami from harmful microbes, i.e., tiny organisms. In biotechnology, molds are also used to produce **enzymes and antibiotics**, among other things. Penicillium, which is responsible for the discovery of the first antibiotic (penicillin), is particularly well known. As a result, they play an important role in medicine and research.

Fungi are therefore not only indispensable for the ecosystem, but also for many areas of industry. They help in the production of food, support biotechnological processes, and provide important medicines. Their ability to break down various materials makes them true all-rounders of nature and valuable helpers to humans.









### **Experiment: mushroom cultivation**

Would you like to grow fresh oyster mushrooms at home? No problem—with a little preparation and the right techniques, you can do it even without any prior knowledge! Here you will learn step by step what you need and how to proceed.

#### What do you need?

Before you start, please read through the entire protocol carefully and have all the materials ready. The following list (Picture 18) shows you what you need for your mushroom cultivation experiment:



Picture 18: Stuff you need to grow mushrooms.

#### Where can you get the materials?

You probably already have most of these items at home, but some are a little more specialized. So here are a few tips: Coffee filters and matching holders can usually be found in well-stocked supermarkets, as can disposable gloves and disinfectant. An empty 1-liter yogurt cup with a lid is best suited as a breeding vessel—it's sustainable and practical. However, you can also use any other container with a capacity of about one liter, a lid, and an opening of at least 7 cm. Just remember: you need to be able to make holes in the lid or the container. Respiratory masks are













now most reliably available in pharmacies. The grain spawn and adhesive filters are a little more special. We ordered both online: the mushroom spawn comes from Pilzmännchen, the adhesive filters from **Biomed Solutions** – we have had good experiences with both products.

#### What other requirements are important?

- A breeding site with a temperature of 20-25°C
- Later, a cooler room with a temperature of 10-20°C for fruiting body growth

#### **Duration of the experiment**

How long it takes until you can harvest the fruiting body depends on the temperature, humidity, and nutrient supply. So, it can take different amounts of time. However, the first growth phases are often completed after **2-3 weeks**. But don't give up if it takes longer.

#### Note

This protocol is suitable for growing **oyster mushrooms** at home. If you already have some experience, you can also try other types of mushrooms.









#### Step-by-step instructions

#### I. Ensure good ventilation

Fungi need oxygen to grow. Prepare your container by carefully drilling or cutting three air holes, each 1 cm in diameter. These should be above the coffee-spawn mixture, either in the lid or on the upper edge. Then cover each hole from the outside with a filter to prevent germs from entering.



Picture 19: A lid with air holes: Lust can get in, but filters keep other germs and fungi out.



Picture 20: A piece of kitchen paper soaked in disinfectant to clean everything.

#### II. Clean and sterilize the cultivation container

Hygiene is essential when growing mushrooms, so wear protective gloves and a face mask when carrying out all steps. Clean the container and lid thoroughly with hot water and washing-up liquid or in the dishwasher. If you want it to be particularly clean, you can boil the materials. To do this, place them in a large pot of boiling water and boil for about 10 minutes. Caution: Be careful not to burn yourself! Ask your parents for help if you are unsure. Then let everything cool down thoroughly. Finally, wipe the parts with disinfectant – preferably with a piece of kitchen paper that is well soaked with it).

#### III. Sterilizing coffee

Always use fresh coffee grounds that are no more than a few hours old. To ensure that they are as germ-free as possible, pour hot boiling water over the coffee grounds again shortly before use. Allow the water to drain, preferably using a coffee filter and funnel. Then pour the grounds into your sterile culture vessel and close the lid. Wait until the coffee grounds have cooled to body temperature.



Picture 21: The coffee substrate is scalded with hot water to kill germs.





Picture 22: This is how much substrate goes into the container SO that the mushrooms can grow well.

#### IV. Mix coffee grounds with mushroom spawn

Put roughly equal amounts of coffee grounds and fungal spawn into the culture vessel. Together, they should fill about 2/3 of the vessel. Mix everything well with a disinfected spoon and close the lid again. If you don't have enough coffee grounds at once, you can add more sterile coffee grounds and mushroom spawn after a few days. Make sure that the substrate is moist but not wet. No water should collect at the bottom.

#### ٧. Wait and let it grow

Place the container in a location with a temperature of 20-25°C and leave it there until the entire substrate is covered with white mycelium. This can take several weeks.



Picture 23: The coffee substrate is completely overgrown with fungal mycelium.



Picture 24: Freezer bags prevent evaporation as soon as the fungus grows over the edge of the container.

#### VI. Temperature change for the fruiting bodies

Once everything is completely white, the mushroom needs a change in temperature to form fruiting bodies. Place the container in a room with a temperature of 10-20°C and leave the lid on until the mushroom has almost grown up to it. You can then place a freezer bag with small air holes over the container and attach it to the rim to retain moisture.

#### VII. Harvesting the mushrooms

Once the mushrooms have grown to a good size, you can carefully cut them off with a knife. You can usually harvest one or two more times from the same batch.



Picture 25: This is what an oyster mushroom looks like when it is ready to be harvested.