

Growing fungal fruit bodies for microscopic examination, part 1

By Veselin Andreev

I've read some articles about mushrooms and I thought that it would be a good idea to write one myself. If you recognize the magnificent inherent features of the mushrooms then you would probably want to find out more about their lifecycle. So why not grow your own mushrooms at home in order to study their developmental patterns. You really don't need any expensive equipment except for your microscope.

So if I've caught your mycological scent then I'll briefly explain to you the basics of indoor mushroom cultivation. The main idea is to germinate spores with compatible genotypes. When a single spore germinates it forms a monokaryotic mycelium. This mycelium will never develop into a mature mushroom because it is also haploid. This means that it has only one set of chromosomes which isn't enough for spore production through meiosis. When two mycelia meet and fuse together (somatogamy) they give birth to a new type of mycelium called dikaryotic. This mycelium is able to grow into the mushrooms we know. It is important to note that only mycelia with compatible genomes can give rise to fruit bodies. There are special cells in the mushrooms called basidia (sing. basidium) and they undergo nuclear fusion (karyogamy) in order to carry out meiosis. They are situated on the gills of the mushroom where the spores are formed. Then the spores detach from the basidium and close the mushroom's lifecycle.



I really don't feel like going into the technical formalities because the net is full of growing info. With a bit of research you can find techniques for all tastes and finances. I personally adore a technique called 'casing'. It is easy to accomplish and looks really beautiful. Basically you cover a carbohydrate rich substrate with a non nutritious layer that is able to sustain high moisture. An oven sterilized mix of peat moss and vermiculite is perfect for the job. If you feel like it you can add crushed oyster shells in order to increase the pH (I've never used it though). However, the first and most important part is to provide absolutely sterile conditions for the mycelium. Any fungal, viral or bacterial contaminations will ruin your experiment.

Vermiculite is biologically non active and it also has great water retaining properties. The rye seed is very nutritious for the fungi since it is a great source of carbohydrates. You mix the rye seed, vermiculite and water in a given ratio and then you sterilize them by using a pressure cooker or something else (again check out the numerous techniques on the net). After you've accomplished

this you need to proceed with inoculation. Inoculation is the introduction of a sterile spore containing

suspension. Check out the quality mycological supply stores and decide what type of mushrooms you would like to grow. There are edible, medicinal and even glow in the dark mushrooms. I suggest that if you are new to mushroom cultivation it would be best to buy a syringe with easy to grow fungi. Follow the supply store instructions.



Mycelium growth seen at the points of inoculation. Note the rope-like strands, which are called rizomorphs. They are a kind of exploratory structures as well as transport channels for metabolites.

You must put your jars somewhere dark and warm. If given optimum conditions the mycelium can colonize the substrate for about a couple of weeks (the speed of colonization varies among species). I am growing *Psilocybe cubensis* and more specifically this particular strain is called B+. There is a lot of controversy regarding its origin and basically nobody knows where it has come from. Some claim that B+ is a hybrid between *P. cubensis* and *P. azureus* due to some common morphological features regarding spores and other reproductive structures. However, I am not aware of any genetic data to back up this assertion. After colonization is complete you can leave your jars for several more days to ensure that the mycelium has colonized even the parts of the grow media that you can't see. So after this step you must decide whether you want to cold-shock your cakes or leave them as they are. A sudden drop in the temperature is like a signal to the mycelium that it is time to stop growing and start producing fruit bodies.



This is a fully colonized rye seed and vermiculite jar. You can clearly see the rizomorphs. After the mycelium has settled-in it is hard for other contaminants to invade the nutritious media.

After you take out the fully colonized cake you must decide whether you want to leave it whole or crumble it to small pieces in order to prepare a casing tray. Basically the whole cake technique is suited for beginners and the casing if for the more advanced growers. However, I failed miserably with my cakes but the casing turned out to be just fine. Probably I should have used better ingredients but the sterilized soil and vermiculite mixture is working well enough for my needs. So in order to prepare a casing you must crumble your cakes into moderate pieces and then cover them with the non-nutritious mixture. I would recommend that you use 50:50 mixture of vermiculite and peat moss and add a small amount of crushed oyster shells if you can find any. You add water to the mixture (check out some internet protocol) and sterilize in an oven for about 4 hours at 180-190C. After the casing mixture has cooled down you can cover the crumbled cakes. The rule is this: the depth of the casing layer is $\frac{1}{4}$ of the whole tray depth. Then you leave your casing somewhere about 28C in a dark place, cover it with tin foil and you've got to mist it daily. When you see the mycelium poking through the valleys of the casing layer stop misting and remove the foil. It is important to avoid overlay (this is

when the whole surface is severely colonized with mycelial strands) but if it happens with *P. cubensis* it is not fatal. Now you have to place your casing in a humid chamber with sufficient fresh air exchange.



This is my perlite humidified chamber. I am having trouble with moisture content in the casing and that's why my chamber looks so wet. However, casings do not require that much humidity since the casing layer acts as a water reservoir. Cakes on the other hand require humidity of around 97%. It is very important to introduce fresh air in the chamber and remove the CO₂ build-up. The carbon dioxide will slow down the growing process.

The process of fruit formation is known as pinning. The formation of primordia (pins=mushroom embryos) is triggered by changes in the atmosphere composition as well as introduction of light with specific wavelength. Ultraviolet and blue waves are perfect for the job whereas green, yellow and red have no effects on the pinning process. Another important factor is the temperature decrease. Generally it is best if you can provide a 10C drop when you take the cakes out of the jars. However, if you are utilizing the casing technique you must give the mycelium some more time to set up a well established network in the upper layers. I believe that patience is the key to success. You shouldn't get frustrated if no pins form at first. You must be on the look out for contamination. There are a lot of bacteria and molds that can take advantage of your experiment. Also there are fungus eating flies that will mate and lay eggs on your mycelium surface. Here is what I found on my tray. I think that it is a larval stage of the fungus eating flies.



A fungus-eating fly larvae. I found this on my tray. I used a drop of 10% formaldehyde solution to immobilize the beast. The magnification in the photo is 10x12,5. I used a mobile phone camera with 2 megapixel resolution and a standard KF Zeiss microscope.

And now you wait for the little mushrooms to appear. However, I had a real problem with the fungus-eating flies and it is very possible that they have brought in a viral or a bacterial infection. Anyway this really didn't turn out so bad since there are a lot of viral and bacterial induced mutations and my tray is covered with bizarre mutant mushrooms. If there are readers who have access to a histological laboratory and are keen on fungi I would be very interested if they use that kind of mutants (they occur really frequently) to prepare permanent slides and compare them to normal mushrooms. Also if you have access to a sterile environment you can make lots of genetic and cloning experiments with those mutants and the normal mushrooms. Here are some pictures of my B+ mutants.





This is my first grow ever and I am very willing to experiment. I hope that mature mushrooms will form soon as I would like to take photos of their reproductive structures (gills, spores etc.). What I want to try is to prepare a spore syringe from a mushroom found in the wild. However, I need really sterile conditions but it's not impossible to accomplish this at home. All you need is a tupperware container with two holes for your hands. And if you succeed in keeping the inner environment clean then you can even clone your favorite mushrooms on agar plates. Subsequently you can prepare a casing tray of genetically identical mushrooms. All in all there is a lot you can do with your mycelia and spores. My advice towards you is to experiment in the field of amateur mycology. Here are some pictures of newly formed mushrooms.



Notice the bluish bruising on some primordia. This means that they don't like my substrate very much. Maybe I should have gone with the peat moss instead of the soil-like substrate.

If you have any problems during your growths feel free to browse around the mycological forums and ask questions. There are some really advanced mycologist that are willing to help you out. Generally my first attempt isn't great but I am pretty sure that persistence and patience will really come in handy.



If you have any comments or questions please feel free to contact me on my email: sswaffe@abv.bg

References:

1. Paul Stamets - 1983 - The Mushroom Cultivator - A Practical Guide to Growing Mushrooms at Home
2. www.mushroomvideos.com (I recommend that you start with the BRF tek)
3. Various mycological forums and portals on the internet