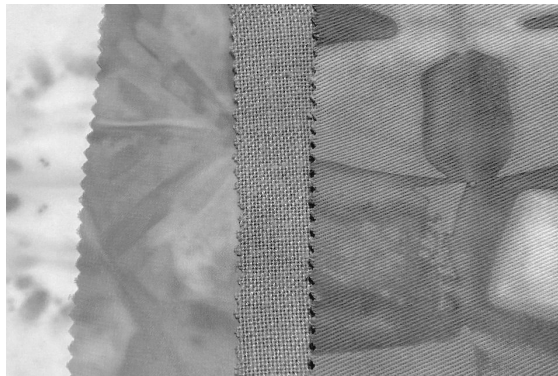


## MICROBIAL DYE

GROWING PIGMENTED BACTERIA ON TEXTILES



SEVERAL MICROORGANISMS NATURALLY PRODUCE PIGMENTS, SOME OF WHICH ARE SUITABLE AS TEXTILE DYES AND INKS FOR ARTMAKING. UNDERSTANDING THEIR NEEDS AND LIFECYCLE WILL ALLOW YOU TO COLLABORATE WITH BACTERIA CREATIVELY.

SEE ALSO: Aseptic technique | Biolab rules | Setting up a community biolab | Morphology of Tools | Organisms to get to know

DIY MICROBIOLOGY

## MICROORGANISMS TO GET TO KNOW

SUPERPOWERED ORGANISMS THAT ARE SUITABLE FOR SCHOOLS



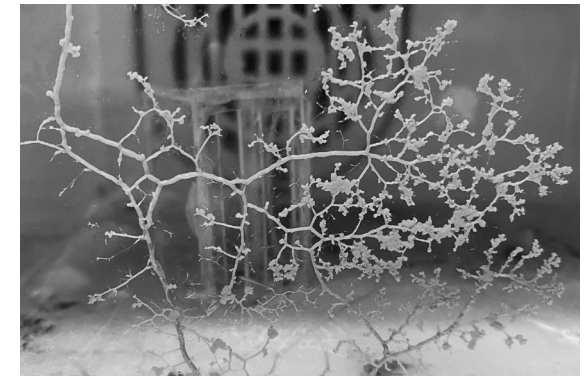
EXPLORING BIOLOGY BUT DON'T HAVE A SCIENCE BACKGROUND? GET TO KNOW SOME FRIENDLY ORGANISMS WITH INTERESTING PROPERTIES TO EXPERIMENT WITH. THE MICROORGANISMS ON THIS CARD ARE BEGINNER-FRIENDLY BEINGS WITH INTERESTING PROPERTIES TO EXPLORE.

SEE ALSO: Setting up a community biolab | Biosafety containment levels | Lab rules

DIY MICROBIOLOGY

## MICROORGANISMS TO GET TO KNOW

SUPERPOWERED ORGANISMS THAT ARE SUITABLE FOR SCHOOLS



EXPLORING BIOLOGY BUT DON'T HAVE A SCIENCE BACKGROUND? GET TO KNOW SOME FRIENDLY ORGANISMS WITH INTERESTING PROPERTIES TO EXPERIMENT WITH. THE MICROORGANISMS ON THIS CARD ARE BEGINNER-FRIENDLY BEINGS WITH INTERESTING PROPERTIES TO EXPLORE.

SEE ALSO: Setting up a community biolab | Biosafety containment levels | Lab rules

DIY MICROBIOLOGY

## BIOLAB RULES

THE IMPORTANCE OF GOOD MICROBIOLOGICAL LABORATORY PRACTICE (GMLP)



GMLP RULES ARE AIMED AT CONTAINING UNCONTROLLED SPREAD OF MICROBES, TO PROTECT YOUR EXPERIMENTS FROM BECOMING CONTAMINATED WITH EXTERNAL MICROBES, AND TO PROTECT YOU AND OTHERS FROM THE POSSIBILITY OF INFECTION.

SEE ALSO: Handwash experiment | Organisms to get to know

DIY MICROBIOLOGY

## HANDWASH EXPERIMENT

WHAT IS LIVING ON YOUR HANDS?



PRACTICE POURING AGAR PLATES AND DISCOVER THE GERMS GROWING ON YOUR SKIN. HAND WASHING THOROUGHLY IS PART OF GOOD MICROBIOLOGICAL PRACTICE BECAUSE IT REDUCES THE CHANGE OF GROWING UNWANTED ORGANISMS (CONTAMINATION).

SEE ALSO: Biolab rules

DIY MICROBIOLOGY

## BIOSAFETY: CONTAINMENT LEVELS

LEVELS OF CONTAINMENT TO ENSURE SAFETY OF PEOPLE AND ENVIRONMENT



Kelsey Chisamore, The Noun Project.

CONTAINMENT IS THE TERM USED TO DESCRIBE METHODS, PRACTICES, PROCEDURES, FACILITIES, AND EQUIPMENT USED TO SAFELY MANAGE BIOHAZARDOUS MATERIALS IN THE LABORATORY.

SEE ALSO: Starting a community biolab | Lab Rules | Aseptic technique

DIY MICROBIOLOGY

# MICROORGANISMS TO GET TO KNOW

NOTE  
In all cases, it is important to learn to identify contamination. When in doubt: do not continue the experiment or open it: sterilize and throw it out.

## TASKS

The organisms marked with an asterisk\* have been identified as suitable use in secondary school labs, are fast growers that aggressively take out competitors (low contamination risk), or grow in acidic environments unwelcoming for most competitors (low contamination risk).

**Janthinobacterium lividum\***  
Janthinus is Latin for violet, which is also the color of the pigment violacein this aerobic bacteria produces when it metabolizes glycerine. This pigment can be used as biodegradable dye for textiles that doesn’t contain the harmful chemicals and heavy metals many synthetic dyes contain.

**Slime mould\***  
Physarum Polycephalum is an a-cellular slime mould that feeds on bacteria and fungi spores (found in e.g. rotting wood). It has “senses” and a primitive intelligence. It can sense wheat and soy nutrients in its environment and has a very efficient way of forming networks for nutrient distribution. It can find the shortest path through a maze and exhibits some form of memory.

**Acetobacter xylinum\***  
Is a bacteria that has the ability to synthesize cellulose from sugars in acidic environments. This biofilm has been used for papermaking, textiles, packaging, wound care and drug delivery systems. Together with other yeasts and bacteria, it is also found in the fermented tea drink Kombucha.

REFERENCE  
• Suitable and unsuitable microorganisms (2018) Microbiology in Schools Advisory Committee (MISAC): [https://www.misac.org.uk/PDFs/MISAC\\_Suitable%20and%20Unsuitable%20Microorganisms2.pdf](https://www.misac.org.uk/PDFs/MISAC_Suitable%20and%20Unsuitable%20Microorganisms2.pdf)

# MICROORGANISMS TO GET TO KNOW

NOTE  
In all cases, it is important to learn to identify contamination. When in doubt: don’t continue the experiment or open it: sterilize and throw it out.

## TASKS

The organisms marked with an asterisk\* have been identified as suitable use in secondary school labs, are fast growers that aggressively take out competitors (low contamination risk), or grow in acidic environments unwelcoming for most competitors (low contamination risk).

**Gray oyster (Pleurotus Ostreatus)\***  
Edible wood-loving mushroom that can be trained to grow on almost anything (straw, coffee, hemp, wood, paper, cigarette buds). Competitors don’t stand much chance against this aggressive fungus, so contamination rates are relatively low, making it great for beginning fungus growers. Spores can cause allergic reactions, search for a sporeless strain (e.g. Homegreen.nl)

**Reishi (Ganoderma Lucidum)**  
Grows slower than oyster mushrooms, but its mycelium is smooth and very strong. Primary decomposer that can thrive even on fresh (not composted) wood substrates (e.g. hydrated mix of 10 parts hardwood saw dust, 2 parts wheat bran, 1 part gypsum). Is less dependent on high humidity and fresh air. Reishi dyes a warm gold beige/rust color with ammonia. Also medicinal.

REFERENCE  
• Suitable and unsuitable microorganisms (2018) Microbiology in Schools Advisory Committee (MISAC): [https://www.misac.org.uk/PDFs/MISAC\\_Suitable%20and%20Unsuitable%20Microorganisms2.pdf](https://www.misac.org.uk/PDFs/MISAC_Suitable%20and%20Unsuitable%20Microorganisms2.pdf)

# MICROBIAL DYE

TOOLS and MATERIALS  
Salt, yeast extract, peptone, glycerine, 70% denatured alcohol, autoclaveable bags, glassware, rubber bands, Fiberfil synthetic wool, micropipette and tips, parafilm. Access to a biolab and plate of Janthinobacterium Lividum BSL-1 teaching strain. Optional: glue clamps, acrylic shapes.

## TASKS

**Prepare the substrate**  
• Apply shibori/tie dye if desirable. Or apply a pattern using liquid latex (you can use a stencil for this too). Place in autoclaveable bags or large petri dishes.

**Prepare a liquid medium and Fiberfil**  
250 g hot water | 0.75 g yeast extract | 1.25 g peptone | 1.25 g salt (NaCl) | 5 g glycerine (1-2% by weight to boost pigment production)  
• Mix the ingredients. Pour onto textile until fully soaked, but without making puddles in the bag.  
• Cut off some pieces of Fiberfil synthetic wool, wrap in aluminum foil.  
• Autoclave everything for 20 mins (<500 ml) or 45 mins (>500 ml), don’t overload the pressure cooker, allow air to circulate. Close bags with clips after opening.

**Inoculate**  
• Allow textiles with medium to cool to 30 degrees C. Inoculate using aseptic technique. When working with a liquid inoculum: use a micropipette. When working with culture on an agar plate, use an inoculation loop.  
• Note: be careful not to burn the bag with the hot loop! Ask someone to assist you with opening and closing the bag.  
• Seal dishes with parafilm and/or plug bags with Fiberfill, then wrap with rubber band to close the bag. The Fiberfil acts as a gas exchange/filter.

**Incubate**  
• Incubate at 22-26 degrees C for 3-5 days or until desired color is achieved. Make sure bags are upright so the culture cannot contaminate the filter.

**Sterilization**  
• Put the bags/plates in the pressure cooker without opening them. Autoclave for 45 mins (creased textile is more difficult to autoclave).  
• Wash thoroughly before drying the textile.

REFERENCE  
• Bioshades (2019) TCBL & Textile Lab Amsterdam: <https://bioshades.bio>

## BIOSAFETY LEVELS

| WHY   | WHEN  |
|---|---|
| Starting to understand how required levels of cleanliness and containment depend on a number of interrelated factors (skill level, protocol and use, volume of culture) enables you to critically assess risks and possibilities. | After you have done some textbook experiments, and are starting to wonder and ideate what else might be possible. |

## TASKS

Discussion prompt 1: Read the biosafety levels manual and discuss the importance of biosafety levels or *levels of containment*. What is the difference with the school levels discussed in the manual? Why do you think they address those specifically?

Discussion prompt 2: Find out under which biosafety level each of these organisms is typically classified (may differ per strain!). Research whether any of these is related to illnesses in humans. Discuss whether you would consider using these organisms in a school biology setting and which conditions you might be set for working with these.

*Pleurotus ostreatus* | *Serratia Marcescens* | *E.coli* | *Komagataeibacter Xylinus*

Discussion prompt 3: why is working in the lab with a Gray Oyster to make materials different from growing these in your kitchen, and different from eating store-bought grey oysters to use for dinner? Why can you eat the mushrooms that you grow in your kitchen (see also Rotterzwam growkits), but you cannot eat mushrooms you grow in a lab where you are also experimenting with other organisms?

REFERENCE  
• Microbiology Society (2016) Basic Practical Microbiology: a Manual: <https://microbiologysociety.org/publication/education-outreach-resources/basic-practical-microbiology-a-manual.html>

## HANDWASH EXPERIMENT

| WHY  | WHEN   |
|--|--|
| Learn why lab rules exist, and what Good Microbiological Laboratory Practice entails, practice with a hands-on experiment. | This is a good introductory activity to familiarize students with key concepts, tools and rules in a biolab, before starting any investigations. |

## TASKS

Study *Basic Practical Microbiology: A Manual* in preparation for class, followed by the handwashing experiment. Students practice pouring plates using aseptic technique, and learn to use the autoclave to sterilize media and materials.

- Prepare a nutrient agar (500 ml water, 1.5 g yeast extract, 2.5 g peptone, 2.5 g non-iodized salt, 7.5 g agar agar)
- Autoclave for 45 mins, allow to cool to 35 degrees Celcius
- Pour agar into sterilized petri dishes using aseptic technique
- Take a bathroom and coffee/tea break until agar sets
- Group 1 washes hands with soap and warm water for 20 sec
- Group 2 washes hands with only water
- Group 3 disinfects hands with hand sanitizer
- Group 4 does not wash or disinfect their hands at all
- Ask each student to press a finger onto the agar, close the dish, seal with parafilm and label it
- Incubate for 2-7 days at room temperature
- Study the results without opening the plates
- Autoclave the plates for 20 mins afterwards

REFERENCE  
• Basic Practical Microbiology: A Manual (2016) Microbiology Society: <https://microbiologysociety.org/publication/education-outreach-resources/basic-practical-microbiology-a-manual.html>

## BIOLAB RULES

| WHY   | WHEN   |
|---|--|
| Good Microbiological Laboratory Practice (GMLP) is one of the main ways to ensure safe practice when working with microorganisms. | This is a good introductory activity to familiarise students with key concepts, tools and rules in a biolab, before starting any investigations. |

## TASKS

Study the manual provided in the reference. Design a poster together, listing all the rules, make it visible in your shared lab space:

- Report spills or damage immediately to a lab technician.
- Only do work you are trained and instructed to do. When in doubt: ask!
- No eating, drinking or hand-to-face contact: may cause accidental ingestion of hazardous materials or culture.
- Label everything, always: so other people are aware of their contents (date, name, organism, growth media)
- Handwashing: before microbiological work to avoid contaminating your experiments with unknown organisms, and after to ensure no living cultures accidentally leave the lab on your hands. Wear a lab coat (polyester/cotton blend) when in the lab.
- Never leave open flames or running pressure cookers: they are potential fire hazards and need to be monitored, always.
- Dispose of waste properly: all living cultures and materials that have been in contact with living cultures need to be steam autoclaved before disposal. Surfaces are to be disinfected with 70% denatured alcohol after use.
- Keep personal items (notebooks, phones, laptops, coats) outside the lab and at all times away from the lab bench.
- You are not allowed to take any living cultures from the or bring in living cultures without permission from a technician.

REFERENCE  
• Basic Practical Microbiology: A Manual (2016) Microbiology Society: <https://microbiologysociety.org/publication/education-outreach-resources/basic-practical-microbiology-a-manual.html>



## SET UP A COMMUNITY BIOLAB

A PROPER ENVIRONMENT PREVENTS CONTAMINATION AND HEALTH HAZARDS



SAFE PRACTICE IN MICROBIOLOGY REQUIRES A DESIGNATED, LIMITED ACCESS SPACE AND TRAINING. A PROPER ENVIRONMENT PREVENTS CONTAMINATION AND HEALTH HAZARDS. THIS CARDS CONTAINS POINTERS TO GET INFORMED BEFORE DOING PRACTICAL WORK.

SEE ALSO: Biosafety Containment Levels | Lab Design | Organisms to Get to Know | Biolab Rules

DIY MICROBIOLOGY

## LAB DESIGN

RECOMMENDATIONS FOR MATERIALS, EQUIPMENT, AND INFRASTRUCTURE



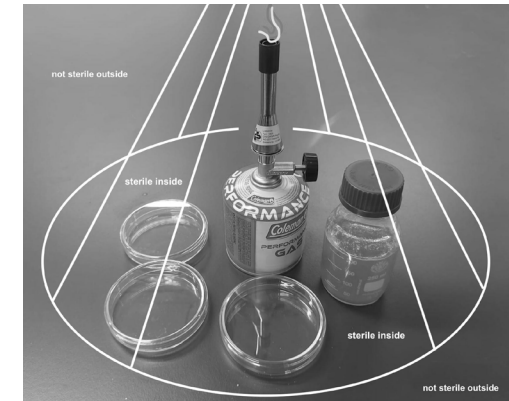
WHETHER SETTING UP A SPACE FOR THE FIRST TIME, OR MOVING TO A NEW SPACE, CONSIDER THE FOLLOWING LIST OF RECOMMENDATIONS FOR MATERIALS TO USE, AND THE INFRASTRUCTURE REQUIRED TO HANDLE THEM WHEN DESIGNING A BIOLAB.

SEE ALSO: Setting up a community biolab | Aseptic technique | Cone of protection | Lab rules

DIY MICROBIOLOGY

## CONE OF PROTECTION

A LOW-TECH STERILE TECHNIQUE FOR WORKING ON AN OPEN BENCH.



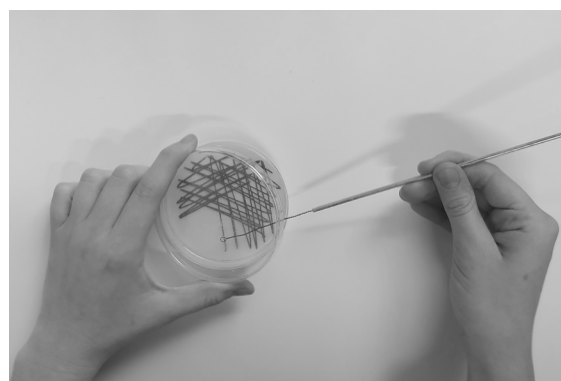
TRANSFERRING ORGANISMS OR INOCULATING PLATES OR SUBSTRATES WITH ORGANISMS NEEDS TO BE DONE IN A CLEAN ENVIRONMENT TO PREVENT CONTAMINATION. WORKING WITH A STERILE BUBBLE IS A STERILE TECHNIQUE FOR WORKING ON AN OPEN BENCH.

SEE ALSO: Aseptique techniques | Lab rules

DIY MICROBIOLOGY

## ASEPTIC TECHNIQUES

POURING PLATES, INOCULATING WITH LOOPS, PIPETTES AND SCALPELS



ASEPTIC TECHNIQUE IS A SET OF PROCEDURES TO PREVENT UNWANTED MICROORGANISMS FROM CONTAMINATING YOUR EXPERIMENTS AND YOUR ENVIRONMENT.

SEE ALSO: Cone of protection | Lab rules

DIY MICROBIOLOGY

## DIY APPLIED MYCOLOGY

COLLABORATING WITH FUNGHI



MYCOLOGY IS THE STUDY OF FUNGI AND THEIR APPLICATIONS IN SEVERAL INDUSTRIES (FOOD, MATERIALS, PIGMENTS, MEDICINE, BIOREMEDIATION). THE AVAILABILITY OF TOOLS AND DIY PROCESSES MAKE THIS FIELD ACCESSIBLE TO ENTHUSIASTS.

SEE ALSO: Set up a community biolab | Aseptic techniques | Mycelium-hemp composite

DIY MICROBIOLOGY

## MORPHOLOGY OF TOOLS

FINDING ALTERNATIVES TO SPECIALIST EQUIPMENT



MANY PEOPLE IN THE DIY BIOLOGY REALM HAVE CONSIDERED WAYS TO MAKE MICROBIOLOGICAL WORK MORE ACCESSIBLE BY FINDING ALTERNATIVES TO EXPENSIVE SPECIALIST EQUIPMENT. SOME TOOLS AND MATERIALS CAN BE SUBSTITUTED.

SEE ALSO: Set up a community biolab | Lab design

DIY MICROBIOLOGY

## CONE OF PROTECTION

TOOLS  
Bunsen burner, lighter, alcohol 70%

### TASKS

The updraft from the heat generated by the Bunsen burner prevents particles in the air from falling into your petri dish. The cold air that is sucked in from beneath comes from the alcohol-covered bench, thus creating a sterile bubble with a diameter of 20-25 cm. Keep the organisms and dishes within the bubble and keep your movements (with a scalpel or inoculation needle) within the bubble.

- Close windows and doors and let everyone know you will be inoculating and lighting the flame
- No talking, no walking around
- Work on a smooth, even and cleaned surface
- Clean everything with 70% alcohol (let it dry on its own)
- Light the Bunsen burner. The blue flame is the hottest (tweak the oxygen supply to change the flame from yellow to blue)
- Work within 20 cm radius of flame
- Don't wear gloves or synthetic face masks (can glue to skin when hot)
- Point tip of alcohol bottle away from flame at all times!
- Open petri dishes as little as possible, open petri dishes towards the flame (open top like a clamshell towards the flame)
- Pass neck of bottle through the flame before and after each pour to sterilize the neck
- Work fast but don't rush, get comfortable
- Don't touch the gas burner when it's on

REFERENCE

- "The Sterile Workspace (n.d.) Neosynbio: <https://www.neosynbio.com/the-sterile-workspace>
- Basic Practical Microbiology: A Manual (2016) Microbiology Society: <https://microbiologysociety.org/publication/education-outreach-resources/basic-practical-microbiology-a-manual.html>

## MORPHOLOGY OF TOOLS

**Glassware**  
You might find that lab grade glassware such as glass bottles can get expensive. Sterilizing media can also be done in glass jam and yogurt jars with a lid. Glass is used because it can withstand the heat of an autoclave (121 degrees C), polypropylene (plastic marked with the sign PP5) which is often used in the production of food containers, is also autoclavable.

**Gas exchange**  
Many microorganisms are aerobic, which means they require fresh air to grow. Others release gases, which can build up in a plate or jar. *Parafilm* is commonly used to ensure gas exchange while providing a barrier for contaminants. Syringe filters, synthetic filter disks (Tyvek) or synthetic wool (e.g. Fibrefill, or non-absorbent synthetic wool) can be used to plug a little air vent drilled into the lid of your jar or bottle.

**Steam autoclave**  
Pressure cooker pans are used in many schools as an alternative. Use of *autoclave tape* is recommended. For steam sterilization to occur, the entire item must completely reach and maintain 121°C for 15–20 minutes with steam exposure at 15 PSI (or 45 mins for textiles, and 500 ml liquids or more).

**Incubator**  
Make your own using instructions from the Biohack Academy program or look for incubators used to hatch reptile eggs.

**Autoclaveable waste bags**  
Invest in different sizes as they can also function as a container for incubated cultures, when closed with rubber band and plugged with a material that serves as gas exchange material (see above). Incubate upright to avoid contamination.

REFERENCE

- <https://learn.freshcap.com/growing/using-pressure-cookers-for-growing-mushrooms/#>
- <https://archermushrooms.co.uk/how-to-make-grain-spawn-jars/>
- [https://github.com/BioHackAcademy/BHA\\_Incubator](https://github.com/BioHackAcademy/BHA_Incubator)

## LAB DESIGN

### CONSIDERATIONS

- Walls and floors should be smooth, impermeable to liquids and easy to clean. No carpets or flammable materials.
- Benchtops should be impermeable to liquids including disinfectants, and chemicals. Benchtops should be scratch-resistant and have no open seams.
- Sink for handwashing, dishwashing and disposal of non-toxic and non-hazardous liquids should be provided.
- Lab furniture such as chairs and stools should be non-porous and easily cleaned (e.g. vinyl, hard plastic, rubber)
- PPE storage such as lab coats should be available upon entry. Lab coats need to be separated, not stacked.
- Personal storage space non-lab items need to be stored outside the lab (e.g. coat racks, closets, lockers).
- Office space is separate from labspace. Demarcate space for eating, drinking and office work
- Fire safety equipment and smoke detectors are often legally required. Fire extinguishers should use carbon dioxide or dry chemical type A-B-C extinguishers.
- Ventilation ideally provides inward airflow without circulation. If mechanical ventilation is not possible, install screens to prevent insects from entering through windows.
- Note: don't make bioplastics inside a microbiology lab, they will get contaminated. Making bioplastics and doing creative work is done elsewhere.

For the Netherlands, see also article 9.1.1.1.1 and 9.1.1.1.2 of the laws regarding work with genetically modified organisms as a guideline for space design: <https://wetten.overheid.nl/BWBR0035072/2021-10-01#Bijlage9>

REFERENCE

- Angela Armendariz, Patrik D'haeseleer and others (ongoing) "Lab Infrastructure & Design" in: Community Biology Biosafety Handbook: <https://bit.ly/3k9Tkz9>

## DIY APPLIED MYCOLOGY

### TASKS

**Set up a community biolab (see related cards)**

- Find suppliers of lab materials (e.g. Eurofysica)
- Learn aseptic technique and Good Microbiological Laboratory Practice (GMLP)

**Choose a well-documented strain**

- Pleurotus Ostreatus (Gray Oyster) and Ganoderma Lucidum (Reishi) are foodsafe strains, suitable for beginners.
- Find a supplier who can sell you *sporeless* strains to avoid unwanted sporulation and allergies (e.g. Homegreen in NL).

**Learn how to grow mycelium in a petri dish (see references)**

- Learn how to make a *malt-yeast-agar* and *potato dextrose agar*

**Learn how to create a grain jar/grain spawn (see references)**

- Learn how to *prepare*, *sterilise* and *inoculate* a grain jar

**Learn how to colonize a bulk substrate to create fungal composites**

- Find out which substrates your strain thrives on (what it likes to eat)
- Learn how to *pasteurise*, *inoculate* and *incubate* bulk substrates
- Learn maintain and dry a bulk substrate

**Learn how to train a strain to digest a particular food**

- Train your mushroom to eat abundant waste, or train it for mycoremediation

REFERENCE

- Peter McCoy (2016) Radical Mycology
- Freshcap Mushrooms Blog and video channel <https://learn.freshcap.com/growing/> and <https://www.youtube.com/c/freshcapmushrooms>

## SET UP A COMMUNITY LAB

**WHY**  
Establishing a shared foundation for bio-safety and security practices is key when you are considering to set up a community lab in your institution or community. Familiarising yourself with resources to do so enables you to conduct safe lab practices with non-biologists.

**WHEN**  
When you want to create an open-access laboratory facility that supports non-biologists such as artists and designers to explore microbiology in a hands-on way.

### TASKS

- Study the *Community Biology Biosafety Handbook* thoroughly with your team (see reference)
- Consult someone with experience as lab technician over seeing practical microbiology work in high schools.
- Find a biosafety advisor who can help with risk assessments (in NL: RI&E).
- Find suppliers of high school lab materials (e.g. Eurofysica, Carolina).
- Learn aseptic technique and Good Microbiological Laboratory Practice (GMLP) by getting training from an expert.
- Write step-by-step protocols defining the acceptable experiments in your lab, review protocols and changes in the future with an expert.

REFERENCE

- Angela Armendariz, Patrik D'haeseleer and others (ongoing) Community Biology Biosafety Handbook: <https://bit.ly/3k9Tkz9>
- Health & Safety (2018) Microbiology in Schools Advisory Committee, UK: <https://www.misac.org.uk/healthandsafety.html>

## ASEPTIC TECHNIQUES

TOOLS  
Bunsen burner, alcohol, scalpel/loop/pipette, agar plates, glass bottle.

### TASKS

Read pages 6-15 from the manual listed below and practice the following techniques. Practice the procedures "dry" (without contents) a few times to get used to the motions.

**Sterilizing tools & Media**

- Steam autoclave all growth media, tools and materials for 20 minutes (45 mins if more than 500 ml liquids). Tools can be wrapped in aluminium foil, so they can be kept closed and sterile until use.

**Pouring plates (aseptic technique)**

- Prepare growth media and autoclave media and petri dishes to sterilize
- Allow to cool until 35 degrees C. The agar sets below this temperature. Agar that is too hot will give condensation inside the petri dish.
- Pour the plates using aseptic technique (p. 13 of manual listed below)
- Wrap poured plates in cling film and store in fridge if not used immediately. Do not use refrigerated agar that is cracked or broken.

**Different inoculation techniques**

- **Inoculation loop:** pass it through the flame before and after every action, ensuring it is red hot. Flame the loop last (start at the base) to prevent aerosol formation of culture.
- **Scalpel:** autoclave before use, then douse with 70% alcohol. Pass it through the flame briefly before each action (not red hot). Used to transfer and inoculate e.g. mycelium.
- **Micropipette:** glass pipettes can be autoclaved and flamed. Plastic disposable tips of micropipettes can be autoclaved for 20 mins inside the box. Keep the box closed as much as possible to keep tips sterile.

REFERENCE

- Basic Practical Microbiology: A Manual (2016) Microbiology Society: pp.6-15. <https://microbiologysociety.org/publication/education-outreach-resources/basic-practical-microbiology-a-manual.html>