Growing mould on bread

Posted by Anonymous on Wed, 2020-04-08 11:23

Growing mould on bread: What are the recommendations, guidelines, and policies relating to students growing mould on food products such as bread?

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Year Level:
Foundation
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Senior Secondary
Laboratory Technicians:
Laboratory Technicians

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Answer by labsupport on question Observing growth on a slice of bread

Submitted by sat on 08 April 2020

Answer revised 1 February 2023

Science ASSIST has developed a comprehensive document called Guidelines for Best Practice for Microbiology in Australian Schools see GUIDELINES for best practice for microbiology in Australian schools. We strongly recommend you download this document as it discusses in detail the underpinning knowledge and laboratory techniques required for schools to successfully prepare, deliver and disassemble microbiology practical activities.

Schools are advised to check what activities are permitted in their jurisdiction/school sector and perform a site-specific biological risk assessment before proceeding to work with any microorganisms.

This activity has a low level of risk, provided safe operating procedures are followed, principally for containment of mould spores. These risks can be well controlled when conducted in the school science laboratory.

Science ASSIST strongly advises against any microbiological experiments being conducted at home as students and their home supervisors are unlikely to have sufficient understanding of the risks associated with microbiological procedures and the need to conduct a biological risk assessment. With this activity we have concerns that there is an increased likelihood that the zip-lock bags may be opened releasing fungal spores and possibly aerosols from other microorganisms that may be present.

What the Australian Standards say

The Australian and New Zealand Standard AS/NZS 2243.3:2022. Safety in Laboratories. Part 3. Microbiological safety and containment, sets out the requirements, responsibilities and general guidelines relating to safe handling and containment of microorganisms in laboratories. It states the following:

> 5.2.3 Work Practices (PC1 Laboratories)
>
> *(d) 'Production of aerosols shall be minimized, particularly where work is carried out on the open bench'.*
>
> *(i) 'Cultures of spore-producing fungi shall be covered or sealed as appropriate to prevent dispersal' 'NOTE 7 Airborne spores can spread in a similar manner to aerosols'*
>
> 11.1 Chemicals
>
> *'Fume cupboards and recirculating fume cabinets shall not be used when working with infectious materials'*
>
> 11.2.5 Respiratory protection

'Microbiological work should be planned to limit the reliance on respiratory protective equipment (RPE). Most laboratory work with microorganisms transmissible to humans by the respiratory route is conducted in containment equipment such as a BSC' [1]

Science ASSIST guidelines on conducting a practical activity investigating mould growth on bread in a school science laboratory: It is permitted to grow and observe mould on bread (also on fruit, vegetables and cheese) in a sealed plastic zip-lock bag or a Petri dish that has been sealed with 4 pieces of sticky tape however, it is **not advisable to open** the bag or Petri dish because of the hazards associated with the release of fungal spores.

Common household microorganisms associated with bread mould include *Rhizopus stolonifera, Penicillium sp., Aspergillus sp.* and *Cladosporium sp*. These are not considered pathogens but are referred to as 'opportunistic'. Opportunistic microorganisms are those able to cause disease if provided with appropriate conditions.

Science ASSIST has produced an SOP: Growing fungi on bread SOP: Growing fungi on bread updated Jan 2023. This activity aligns with **School Work Level 1** in the Science ASSIST microbiology guidelines. School work level 1 is considered very low risk due to the type of microorganisms used and the activity performed. Under this level, bread mould can be grown and observed in closed containers which are never opened.

Conducting activities beyond simple observation of bread mould in a sealed container requires a high level of staff training in microbiology and specific laboratory facilities. Most school laboratories are classified as Physical Containment Level 1 (PC1), if they conform to the requirements set out in Section 5 of the Australian and New Zealand Standard AS/NZS 2243.3:2022 Safety in Laboratories. Part 3. Microbiological safety and containment. This means they are suitable for work with risk group 1 microorganisms, where the hazard levels are low and no special containment equipment is required.

Main hazards, safety measures and PPE required for this activity

- The release of airborne fungal spores (microbial aerosols) is the main hazard to consider during this activity. The microscopic spores float in the air and disperse throughout the laboratory where they are likely to stay for up to 4-5 weeks (M. Cole, personal communication, 12 April 2019). People who suffer from asthma, allergies or are immunosuppressed, may be more sensitive from exposure to airborne fungal spores.
- Bread or other food samples (fruit, vegetables or cheese) should be placed into zip lock bags or Petri dishes which should remain closed throughout the activity and arenever opened to avoid the spread of fungal spores into the air. Growth should only be viewed in the unopened containers in which they were grown.
- Given that there are several different groups of teachers and students that may use the same science laboratory, the risks of adverse reactions extend beyond the staff and students who originally conducted the activity.
- Depending upon the presence and type of air-conditioning systems, these may spread the fungal spores to rooms beyond the laboratory where the activity was conducted.
- Torn/leaking bags: A spill will also lead to spores being released and the need to disinfect the contaminated surface.
- Mould growing on bread or other food samples should never be subcultured as the identity of any mould and microorganisms present will be 'wild' unknown cultures some of which may be pathogenic.
- There is no need to drop the bread onto various environmental surfaces. Simply placing the bread or other items of food (fruit, vegetables or cheese) into a zip-lock bag or Petri dish with a little moisture and incubating in a warm location should be sufficient to allow for the growth of moulds.

- Environmental surfaces if sampled should not be from areas that are likely to contain pathogens such as toilet areas, human body fluids or skin and surfaces where raw meats are handled.
- Wear appropriate PPE, such as safety glasses. Consideration should be given to the wearing of aprons and face masks for high-risk individuals, in the unlikely event of a spill when examining the samples, to provide protection from aerosols.
- No hand-to-mouth operations should occur such as chewing pencils, licking labels.
- All exposed cuts and abrasions must be protected with suitable waterproof dressings before starting practical work.
- There should be no eating or drinking in the science laboratory.
- Hands should be washed with soap and water after the activity and before leaving the laboratory.

Using double bags: Using 2 plastic zip-lock bags helps minimise the risk of leakage and release of aerosols. However, using two bags may make it more difficult to observe any growth of mould. If you were to use a single bag, then sourcing and purchasing a good quality bag would be important.

Incubation times: The incubation time for the mould cultures to grow will be dependent upon the growth conditions provided. Moulds grow best in warm, dark and moist conditions. Consideration needs to be given to the local climate and/or weather conditions. Moulds flourish in warm humid conditions and Northern Queensland fits this scenario during the summer months. In Tasmania, it can take up to two weeks for mould to grow even in summer.

In the school science laboratory moulds can be incubated in a dark cupboard at room temperature or in an incubator set at no higher than 30oC. Incubation time can range from several days to weeks. Daily monitoring would need to be done to determine when mould growth is optimal. Placing the samples in a fridge that is designated for laboratory samples and does not contain food for human consumption is a good idea to slow down the growth of the mould. However, it must be understood that the mould will continue to grow under these low temperature conditions but at a slower rate.

Anaerobic organisms: Anaerobic bacteria, or anaerobes, are bacteria that do not need oxygen to survive. Some anaerobes are beneficial to humans, but others are pathogenic.

Placing bread in zip-lock bags should not encourage the growth of anaerobic organisms. Bread is not a selective medium for isolating these organisms. Bread can occasionally spoil due to bacterial growth, either by the growth of bacilli from spores that survive baking, or by contamination with *Serratia marcescens* which may turn the bread red. The presence of various inhibitory substances/preservatives in many types of bread keeps them from spoiling. Cheese, vegetables and fruit samples would also not be considered as selective medium for growing anaerobic organisms.

If placing bread into Petri dishes, ensure that the lid and base of the Petri dish is taped with 4 pieces of sticky tape[2, 3] to allow for aerobic conditions and to prevent accidental opening of the plate during incubation. Alternatively, one piece of laboratory sealing film e.g., Parafilm® M that is cut no wider than 1cm may be wrapped **once** only around the circumference of the agar plate to allow adequate gas exchange. Gas permeability data for Parafilm® M indicates that when used as a **single layer it will allow sufficient oxygen exchange** to promote the growth of aerobic microorganisms and inhibit the growth of potential anaerobes.[4]

Subculturing: The aseptic transfer of microorganisms from one medium to another is a specialised technique requiring sound knowledge and expertise to minimise the risks involved. It is a skill developed with much practice. This procedure is not permitted in some jurisdictions. You should check the activities permitted in your jurisdiction before proceeding. Teachers supervising students carrying out these activities should be highly trained in microbiological techniques.

Subculturing should only be conducted using pure cultures of RG1 microorganisms. Students should never subculture from cultures that they have inoculated because of the risk of contamination with unknown microorganisms.

Preparing slides for microscope viewing: It is technically difficult to prepare slides of mycelium and spores for microscope viewing.

- It is most likely that students will create a slide with a mass of material on it which is tangled, and it will not be possible to discern any structures.
- Even experienced mycologists may have to prepare many slides before one is clear enough to identify the structures of a fungus.
- The manipulation of fungal cultures with forceps and needles also generates fungal spore aerosols adding to the load in the laboratory environment.

It is recommended that alternative practical activities are provided for students such as the use of:

- A **Dissecting microscope or magnifying glass** to view mould on bread or other fruits or vegetables through the lid of a sealed Petri dish or zip-lock bags.
- A **Compound microscope** to view purchased prepared slides of the same or similar moulds.
- A **BioViewer** to view photomicrographs of the same or similar moulds. A BioViewer is an instrument like a microscope which requires no power source, batteries or light source. Ambient light is utilised to magnify purchased photomicrographs.
- **Food based 'wet' microorganisms** for school microbiology activities, examples include yoghurt or wine or baking yeast in wet pack. If dry it will need rehydrating for 30mins prior to using.

Spill cleanup: If a spill occurs during the experiment (bags leaking or tearing), students must report this to their teacher immediately. Care should be taken as aerosols of spores are likely to be released during a spill. If this occurs, it may be advisable to exit any students from the laboratory that may be at risk due to asthma, allergy or immunosuppression. Benches/floors should be wiped with a suitable disinfectant (e.g.,1% v/v solution of sodium hypochlorite, or 70% v/v ethanol or methylated spirits). Both have good activity on mould. Wear disposable gloves and mask to decontaminate the area.

Waste disposal: Do not open the zip-lock bags or Petri dishes. Bread slices or other food samples should remain in the closed bags or Petri dishes when the activity is complete. These are then double bagged and disposed of in the bin, as normal household waste. Clean laboratory benches and all used equipment (such as knives and chopping boards) with hot, soapy water.

Fume cupboards and Biological Safety Cabinets: Fume cupboards, laminar flow cabinets and biological cabinets work differently for different purposes and differ in the level of protection provided to the user.

- Fume cupboards are not designed for biological work.[1] They operate differently and are designed for use with chemical hazards, such as hazardous gases, vapours, fumes and dusts. They draw air away from you and protect you, the worker from chemical hazards.
- A Laminar flow cabinet directs air across the workspace and towards the user and the laboratory. It protects the specimen being used from contamination and offers no protection for the user or environment from any infectious materials/aerosols.
- A biological safety cabinet (BSC) depending on the class (I, II or III), can protect the user, the environment and the specimen being handled from contamination. Air is HEPA-filtered before release back into the environment.

References

1 Reproduced by ASTA with the permission of Standards Australia Limited under licence CLF1222asta

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