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Footprints in the agar: growing bacteria from ants' feet to combat plant diseases

Teacher instructions

Making PDA culture plates

For these experiments potato dextrose agar (PDA) is used. PDA is a very common media used in microbiology, and all of the agar used in these activities is PDA. It can be bought already prepared to save time, but below is a guide on how to make it yourself.

Notes

- Microbiologists usually refer to agar-filled Petri dishes as 'agar plates' or 'culture plates'.
- Agar plates should be made at least 3 days before use, so that contaminated plates can be spotted and discarded. The agar plates can be stored in a fridge at 5°C for 2–4 weeks, or at room temperature for a maximum of 2 weeks.

Materials

- Potato dextrose broth or PDA
- Milli-Q water or demineralized water
- Autoclave
- 1 l glass bottles + lids
- Petri dishes

Procedure

- Follow the measures on the box (this is usually 24 g L^{-1} for potato dextrose broth and 39 g L^{-1} for PDA). If using broth, add 15 g of agar.
- Add the measured amount of potato dextrose broth/agar to the bottle.
- Add 1 L of Milli-Q water or demineralized water to the bottle.
- Shake until dissolved.
- Sterilize by autoclaving at 121°C for 15 min.
- Once cooled to around 50°C, pour the agar into Petri dishes.
- When the agar plates have set, store in plastic bags (or the bag the Petri dishes came in) with the lid facing down, at room temperature or in a fridge (around 5°C).

Making autoclaved water

Autoclaved water for Activity 2 is made as follows:

Procedure

- Fill a glass bottle with demineralized water or Milli-Q water, and close the lid.
- Sterilize by autoclaving at 121°C for 15 min.
- After autoclaving the water, it can be stored with the lid screwed tightly on for several months.

If students are to inoculate their own plates with fungi, you can make multiple smaller bottles of sterilized water, so that each group gets their own bottle.

How to grow *B. cinerea* or *F. graminearum* from a culture

Botrytis cinerea and *Fusarium graminearum* can be bought as a growing culture through websites like DSMZ.de. Once the cultures have been bought, they should be inoculated onto fresh plates. If students are to inoculate their own plates for Activity 2, you should make one pathogen plate to make the inoculum per group. This should be done at least 3 days prior to starting the experiment. When growth is visible on the plates, they can be stored in a fridge for 2 weeks.

Materials

- PDA plates
- Inoculation loops
- *B. cinerea* or *F. graminearum* culture
- Nitrile gloves

Procedure

1. Wearing gloves, open the vial containing the growing *B. cinerea* or *F. graminearum* culture, and use an inoculation loop to scrape spores and hyphae from the culture.
2. Spread the scraped off hyphae and spores onto clean agar plates, preferably as a three-way streak.
3. Inoculated plates are kept at room temperature for 2–3 days until growth is visibly covering most of the plate.
4. When growth is visibly covering the plates, put them into a plastic bag, lid facing down, and store them in a fridge at approximately 5°C until use.