How to plate conidia and ascospores.
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Background

One may wish to plate either type of spore for a number of reasons and as long as the greater sensitivity of conidia to heat is taken into account, a similar approach can be used for both. Spores can be plated with a sterile spreader or using molten agar, with this latter method typically yielding a more even dispersal.

Irrespective of how spores are plated, a method that ensures colonial growth is an essential adjunct (see accompanying method *How to obtain colonial growth for platings*). The method used to restrict lateral growth will determine how many spores should be plated. For instance, 300 to 400 *cot-1* ascospores per plate is quite reasonable if plates spend no more than 8 or so hours below the restrictive temperature, but a similar number of spores on a plate with sorbose as the restricting agent will yield confluent growth.

Procedure

Spread-plate technique

If no spreader is available then one can be easily made from a Pasteur pipette. Seal the end of the pipette in a Bunsen flame and use gravity to form the bends after heating the appropriate region of the pipette.

Sterilise the spreader by dipping in alcohol and flaming then allow to cool briefly. Transfer a drop of spore suspension (50 to 100µl) to a suitably supplemented plate and while rotating the plate, move the spreader back and forth briskly until the liquid is absorbed.

Plates containing conidia can be incubated immediately but ascospores need to be activated prior to incubation. We heat-activate ascospores by incubating plates at 60°C for 60 min. (see the accompanying method *How to activate ascospores* for other approaches**)**.

Molten agar

The following volumes are appropriate for 8.5cm Petri dishes. Spores can be added to molten agar before plates are poured (20 to 25ml of 2% agar) or afterwards as a top layer on existing solid medium (3 to 5ml of 0.8% agar).

The time that that spores spend in molten agar and its ideal temperature depend upon which type of spore is to be plated. Ascospores should spend 45 to 60 minutes in agar held at 60°C prior to plating (for heat activation) while conidia, which are killed by excess heat, should spend as little time as is possible in agar held at 48 to 50°C.

For ascospores: add the spore suspension to molten agar at 60°C, hold at 60°C for 45 to 60 min. and dispense into or onto the plate.

For conidia: add the spore suspension to molten agar at 48 to 50°C, and dispense into or onto the plate immediately.

Plates can then be incubated at the appropriate temperature.