Isolation and Identification of Fungi from a Clinical Specimen

Brief Summary

You will be provided a nail clipping from an individual having onychomycosis – a fungal infection of the nail. From this clipping, you will isolate a fungus in pure culture, then attempt to identify it based upon morphological features. This isolate will also be used as a source of DNA for performing both a molecular-based identification and a phylogenetic analysis.

Procedure

1. A piece of nail clipping will be provided to each student in a Petri dish. Using aseptic technique, place the nail clipping on the inside portion of a second Petri dish lid (sterile). Using a sterile scalpel and a sterile forcep, carefully chip away at the material to produce dust-like "flakes" across the Petri dish lid. Return the nail clipping to its original container.
2. Obtain a Petri dish of Sabouraud's dextrose agar (SDA) medium that also contains ampicillin (100 µg/ml) streptomycin (100 µg/ml), and chloramphenicol (33 µg/ml). Remove the lid and replace it with the one that contains the nail clipping "flakes". Gently tap on the lid to cause the flakes to fall onto the SDA surface. Replace the original lid, then incubate the plate at 25°C (room temperature).
3. Repeat steps 1 and 2, but use a plate potato dextrose agar (PDA) containing ampicillin (100 µg/ml), streptomycin (100 µg/ml), and chloramphenicol (33 µg/ml) in place of the SDA medium.
4. Periodically over the next week, inspect your plates looking for filamentous growth or the development of yeast colonies. You may wish to use the dissecting microscope to closely inspect your samples for fungal growth at the edges of the "flakes". When appropriate (i.e., prior to becoming cross contaminated with another bacterial or fungal colony), use a sterile scalpel and needle, or a microbiological loop if a yeast colony, to transfer the fungus to another Petri dish containing either PDA or SDA (without antibiotics). Incubate this culture at 25°C allowing it to grow until you are sure that it is pure, i.e., not contaminated with bacteria or other fungi.
5. When you are certain that your culture is pure, aseptically transfer a portion of the colony to a test tube slant of PDA or SDA. Incubate this slant culture at 25°C. This culture will serve as your stock culture that should be periodically transferred to a fresh slant (approximately every 2-3 weeks).
6. The remaining material in the Petri dish culture from step 5 can be used to directly examine the isolate using a tape touch technique (Harris, 2000) and by preparing a slide culture (Harris, 1986). Follow the instructions provided in these references. These two articles are available on the web page for this laboratory.