A method for physical removal of fungal contaminants from established algal cultures.

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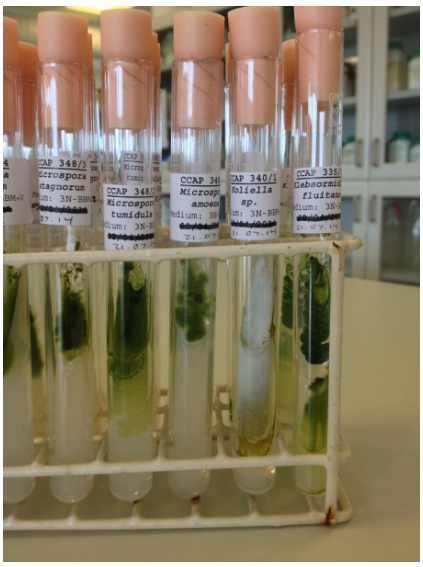
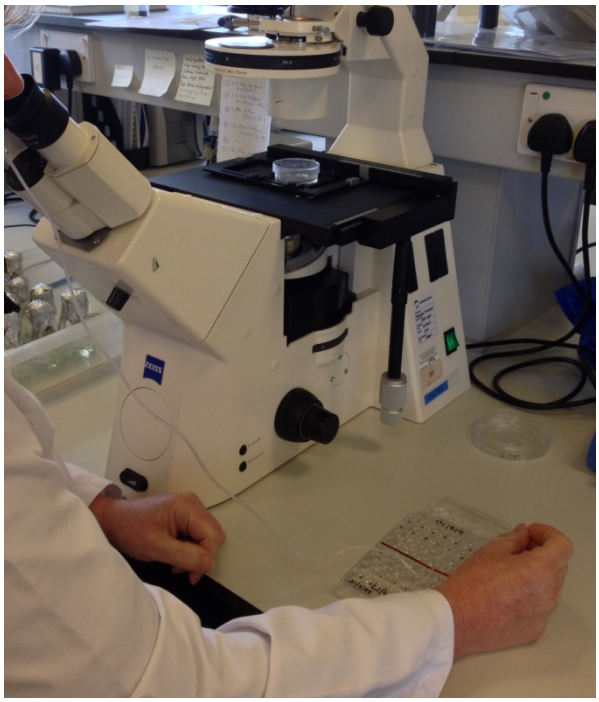


Established strains of microalgae regularly maintained in laboratories often acquire fungal contamination. Excellent aseptic technique should prevent this, however, human behaviour will ensure that it occurs from time to time. Chemical removal of fungus is problematic due to the harshness of the agents and user safety. 100% elimination is difficult to achieve and there is a danger that the anti-fungal agents select a subset of algae which may differ genetically from the original strain, hence physical removal is the method of choice.

METHOD

Ensure all equipment is sterile prior to initiation of the procedure and maintain the highest possible standard of aseptic technique throughout.



1. If the culture is maintained on agar as above, first inoculate the strain into liquid medium and leave it to grow up for 1-2 weeks.
2. Ultra-sonication can be used to break up cells in clumps, or surrounded in extracellular mucilage, before attempting to isolate individual cells.
3. Reduce the number of contaminants in the culture by:
   a. Centrifuging 10-15mls of sample at low speed (~100 x g or 2000rpm in a bench top centrifuge) for 5 minutes. Decant and discard the

Algal culture: Liquid culture of fungal contaminated strain

Apparatus: Bench centrifuge; 15ml centrifuge tube ;20µm and 50µm sterile filters; inverted microscope with low magnification objective (x5, x10) or stereomicroscope; glass capillary tube attached to 0.5m silicon tubing, sterilised by boiling; 48 well plates.

Culture medium: filtered to sterilise and remove detritus.
(see www.ccap.ac.uk/media)

Micropipettes: Blaubrand 20 microlitre and 50 microlitre capacity, 125 mm long or haematocrit tubes

Plasticware: 48-well plates and 35 mm Petri dishes filled with culture medium.
Celltrics® sterile filters, mesh size 10,20,30,50,100 and 150 microns, available from Partec (www.partec.com)

All chemicals were purchased from Sigma-Aldrich, unless otherwise stated.

Additional information:

Andersen RA and Kawachi *(2005) Traditional Microalgae Isolation Techniques. In Algal Culturing Techniques ed Andersen RA. Academic Press.*

culturecollection
of algae and protozoa

supernatant, then resuspend the pellet in sterile dilute culture medium. Repeat 4-5 times.

b. Wash the sample with sterile medium through a filter of a suitable mesh size (e.g. use CellTrics 20 micron mesh to wash algal cells > 25 microns diameter). Place sterile filter in the neck of a test tube or beaker, pipette medium over the cells and discard the filtrate. Repeat 4 - 5 times. Connecting the end of the Celltrics filter to a syringe through a short piece of silicon tubing speeds up the washing stage as the filtrate can be sucked out directly into the syringe. Finally, invert the filter and wash the cells into fresh sterile medium in a small Petri dish.

4. Dilute the sample in filtered medium so that it is easy to pick up individual cells using a micro-pipette.

5. Check microscopically to check that there is no obvious remaining contamination and there are algal cells visible. Inoculate into sterile medium in the wells of the 48-well plate. Repeat the process with as many individual cells as you can pick out, then seal the plate with Parafilm^{TM} and incubate in a moist illuminated incubator for 2-3 days before checking for growth of pure cultures.

6. After a period of time with frequent microscopic examination it will be possible to transfer from the well to larger volume cultures. Subsamples should be taken, inoculated into enriched medium and incubated in the dark to check for the presence of fungus.