Maintenance of marine heterotrophic flagellate protists

Undine E.M. Achilles-Day & John G. Day
Scottish Association for Marine Science (SAMS), Oban, UK

PREAMBLE. The methods outlined below are based on those routinely employed at the Culture Collection of Algae and Protozoa (CCAP).

Flagellated heterotrophic microeukaryotes have key roles in the functioning of marine ecosystems as they channel large amounts of organic carbon to the upper trophic levels and have a major role in the control of the population sizes of both eubacteria and archaea. Phylogenetically it is apparent that this evolutionary heterogeneous assemblage comprises a huge diversity of taxa.

Protists: Monoxenic flagellate culture.

Apparatus: a class I biological safety cabinet; temperature controlled incubator; inverted microscope with 100-500 x magnification equipped with phase-contrast and bright field optics.

Cell culture medium: ASWP, or other appropriate medium. Sterile filtered seawater.

Plasticware: Petri dishes (5cm diameter); tissue culture flasks; sterile disposable pipettes; sterile loops.

Chemicals used routinely are of Analar grade purchased from Sigma-Aldrich, unless otherwise stated.

Additional information:
***Day JG, Achilles-Day UEM, Brown S & Warren A (2007)** Cultivation of algae and protozoa. In: manual of environmental microbiology. Hurst CJ, Kudsen GR, McInerney MJ, Stezenbach LD & Walter MV (eds). ASM Press, Washington DC. pp 79-92.*

1. Prepare and sterilise all media in advance, aseptically decant sterile medium into Petri dishes (5cm diameter) adding 10-15 ml per dish, or tissue culture flask (50 ml), adding 15-20 ml per flask. For virtually all strains a surface sterilised (by boiling for ~1 min) grain (wheat, barley or rice) should be added to medium.

   Choice of culture vessel may vary between strains and resource availability. In general cultures in tissue culture flasks remain viable for longer and are more suitable for transportation. Petri dishes are significantly cheaper.

   Choice of medium will be dictated by the origin of protist, but most isolates originating from oligotrophic environments generally "perform better" in sterilised seawater; most coastal derived strains grow well in ASWP:

   http://www.ccap.ac.uk/media/documents/ASWP.pdf

   Addition of a surface sterilised, previously dry, grain ensures a slow release of carbon/nutrients into the medium, thus ensuring steady growth of "food" bacteria. If possible grains that have not been treated with any pesticides etc. should be used. Choice of grain does not appear to be crucial and wheat is used for most strains at the Culture Collection of Algae and Protozoa (CCAP).

2. Select a dense culture from existing stocks. The state of a culture is ascertained by examination of the culture using an inverted microscope (400 -500 x magnification) under phase contrast.

   In general cultures where actively motile trophs are readily observed will be selected as an inoculum. Note cell densities for heterotrophic flagellates in the standard culture systems may vary from <10 cells ml^{-1} to > 1 x 10^4 cells ml^{-1}. Additionally, for some

isolates large amounts of detritus/bacteria may obscure the presence of the protists. Most marine flagellate cultures remain stable for considerable lengths of time and cultures chosen to provide the inoculum for sub-culture will normally be 10-15 weeks old.

3. To sub-culture, using standard aseptic technique dislodge any flagellates adhering to the biofilm at the bottom of the culture vessel (for tissue culture flasks invert several times; for Petri dishes gently scrape the bottom of the dish using a disposable plastic pipette). Using a disposable pipette aseptically transfer ~2 ml of the suspension to the new culture vessel.

   Always ensure that the Petri dishes or tissue culture flasks are fully labelled with the organisms' name, strain designation, date of inoculum and medium prior to transferring the inoculum.

   The food source for the flagellates is usually the (unidentified) bacteria with which the strains were isolated originally. These bacteria are co-transferred each time a strain is sub-cultured, and multiply on the agar surface.

4. To minimise evaporation over time, seal the junction between the lid and the base of each plate with a narrow strip of Parafilm^{TM}.

5. Incubate inoculated cultures static, in the dark or low light in an appropriate incubator under a controlled temperature regime.

   For most marine taxa 15^{o}C is the most appropriate temperature; however for polar isolates lower temperatures (5-10^{o}C) may be optimal.