Maintenance of marine amoebae

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

Amoebae are extremely common in marine environments and can be easily isolated from most coastal niches, often being particularly prevalent in rock-pools, or associated with living, or decaying, seaweed. Many taxa found in estuarine systems are extremely osmo-tolerant and grow in media ranging from full seawater to those appropriate for freshwater isolates.

Maintenance on agar "washing" method

1. Prepare and sterilise all media in advance, aseptically decant sterile agar into Petri dishes (9cm diameter) and after gelation store, close Petri dishes at room temperature for a minimum of 72 hours to check for any inadvertent contamination during preparation.

   Choice of medium will be dictated by the origin of protist, but most isolates originating from seawater will grow better and remain stable for longer in a medium based on 75% seawater such as MY75S:

   http://www.ccap.ac.uk/media/documents/MY75S_updated.pdf

2. Select a dense culture from existing stocks. The state of a culture is ascertained by microscopical examination at x 50 magnification, looking at the surface of the agar for actively dividing amoebae.

   Usually, a three week old culture is chosen to provide the inoculum for sub-culture.

3. To sub-culture, using standard aseptic technique dislodge the amoebae from the inoculum culture by repeatedly 'washing' the surface of the agar with an approx. 0.75ml of sterile 75% (v/v) seawater, using a sterile Pasteur pipette. Distribute the resultant suspension of amoebae across the surface of two new agar plates in series of longitudinal 'streaks'.

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

Protists: Preferably monoxenic amoeba culture, or environmental sample containing significant numbers of the target protists. For some applications a food bacterium is required, e.g. a non-pathogenic Escherichia coli.

Apparatus: a class I biological safety cabinet; temperature controlled incubator; inverted microscope with 50-100 x magnification equipped with phase-contrast and bright field, scalpel.

Cell culture medium: MY75S, or other appropriate medium. Nutrient agar for growing food organisms if required.

Plasticware: Petri dishes (9cm diameter); 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.*

Page FC *(1983) Marine Gymnamoebae. Institute of Terrestrial Ecology, Cambridge, 54pp.*

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The food source for the amoebae 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.

Alternative procedure involving addition of food-bacteria:

Prepare the MY75S plates in advance by transferring food bacteria (*Escherichia coli*), with a loop to a new plate and applying it as a lawn onto the agar surface. [*E. coli* is grown as a lawn on Nutrient agar, each MY75S plate requires ¼ of an *E. coli* plate].To subculture the amoebae use the procedure outlined above and place the excised block amoeba-side-down onto one end of the *E. coli* streak. In the same way, a second block is cut and transferred to a second plate. Then treat as detailed below.

4. To minimise dehydration of the agar, seal the junction between the lid and the base of each plate with a narrow strip of Parafilm™ or Clingfilm™.

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

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

Maintenance on agar using the "agar block inversion" method

1. Prepare and sterilise all media in advance, aseptically decant sterile agar into Petri dishes (9cm diameter) and after gelation store closed Petri dishes at room temperature for a minimum of 72 hours to check for any inadvertent contamination during preparation.

   Choice of medium will be dictated by the origin of protist, but most isolates originating from seawater will grow better and remain stable for longer in medium based on 75% seawater such as MY75S:

   http://www.ccap.ac.uk/media/documents/MY75S_updated.pdf

2. Select a dense culture from existing stocks. The state of a culture is ascertained by microscopical examination at x 50 magnification, looking at the surface of the agar for actively dividing amoebae. Using a marker pen, mark by dots on the base of the plate two areas with dense growth of amoebae at the edge of the advancing growth front.

   Cultures should be sub-cultured/ inoculum should be selected before the amoebae cover the whole surface of the agar, for most strains this is usually when they are three to six weeks old.

3. To sub-culture, excise a small block of agar (about 9 mm^2) from above one of the marked dots using a flame-sterilised scalpel (previously placed in 100% ethanol). Aseptically transfer to a new agar plate and placed it amoeba-side-down about 2 cm from the edge of the agar. Repeat this step for the second block excised from the original agar plate.

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

   The food source for the amoebae 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.

Alternative procedure involving addition of food-bacteria:

Prepare the MY75S plates in advance by transferring food bacteria (*Escherichia coli*), with a loop to a new plate and applying it in a Z-shaped streak onto the agar surface. [*E. coli* is grown as a lawn on Nutrient agar, each MY75S plate requires ½ of an *E. coli* plate].To subculture, the amoebae use the procedure outlined above and place the excised block amoeba-side-down onto one end of the *E. coli* streak. In the same way, a second block is cut and transferred to a second plate. Then treat as detailed below.

4. To minimise dehydration of the agar, seal the junction between the lid and the base of each plate with a narrow strip of Parafilm™ or Clingfilm™.
5. Incubate inoculated plates static, in the dark or low light in an appropriate incubator under controlled temperature regime.

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

Preparation of *Escherichia coli*

1. Using a sterile cotton wool swab, the bacteria from an established culture are spread over the entire surfaces of nutrient agar plates. The plates are incubated at 20°C, for 1-2 weeks, until required.

If *Escherichia coli* is required in a shorter time-frame it can be grown at 37°C and sufficient material will be available within 24-48h.