CULTURING OF *PSEUDO-NITZSCHIA* SPECIES ON AGAR-F/2 MEDIUM

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Algae: Method has been successfully employed for Pseudo-nitzschia species

PREAMBLE. The method outlined below was employed for *Pseudo-nitzschia* species. The long-term cultivation of this pennate diatom in liquid media is difficult and often unsuccessful. The growth on agar plates can be considered an alternative method which ensures a reduction of operational time and good results in terms of survival/shape of the cultures.

1. **Preparing of 1 l of agar-f/2 solution (at 0.2% of agar)**

   Add 2 gr of agar to 1 l of seawater.

   Sterilize by autoclave.

   Add stock solutions as reported in the recipe for F/2 medium in Guillard (1975).

   N.B. *Add the stock solutions when the agar has still not reached the gelling point.*

   Prepare 6-wells plates with 4 ml of agar-f/2 at 0.2%.

   Bring the plates at room temperature, seal them with a strip of Parafilm™ to minimise dehydration of the agar. The cultures should be inoculated the next day.

Apparatus:

Solutions: F/2 medium, agar

Plasticware: 6-wells plates, loops

2. **Plating**

a) From cultures maintained in liquid medium to agar:

   Select a dense, healthy culture in exponential growth phase. Add 0.2 ml of the culture and 1ml of f/2 medium to an agar-well and mix it with a loop to be sure that the cells are homogenously mixed into the agar.

b) From agar to agar:

   Add 0.5 ml of f/2 medium to the culture on agar to be transferred and mix it with a loop to homogenise it. In this way, the agar medium becomes softer and favours the success of culture transfer.

Additional information: Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. pp 26-60. In Smith W.L. and Chanley M.H (Eds.) Culture of Marine Invertebrate Animals. Plenum Press, New York, USA.

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Using the loop pick some material (microalgae and agar) from the old plate and mix it in the new agar-well.

Repeat this step for 4-5 times for the same culture strain, to be sure that enough material is transferred in the agar-well.

Add 1 ml of f/2 medium and mix again.

Seal the plate with a strip of Parafilm^{TM}.

Keep the cultures at 12:12 L:D cycle; 18-20°C and 50 µmol photons m^{-2} sec^{-1} of irradiance. The temperature can vary depending from the sites where strains have been isolated.