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Axenic Diatoms cultures protocol V.3

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Protocol status: Working
We use this protocol and it's working

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Abstract

Axenic cultures protocol

Axenicity of cultures is obtained by multi-antibiotic treatment: by adding antibiotics directly to the medium in which the strain will be inoculated. After a variable amount of days we refresh the coltures with new medium.

Troubleshooting

antibiotics concentration

1 The antibiotics used are streptomycin, penicillin and ampicillin;
We used the following final concentration:
- Streptomycin 0,5 mg/ml
- Penicillin 0,05 mg/ml
- Ampicillin 0,03 mg/ml

2 Add antibiotics in F/2 medium.
Start from 20/50.000 cells in 25 ml of F/2 medium with antibiotics.
Refresh 3 times the cultures every 3 days with new medium with antibiotics.

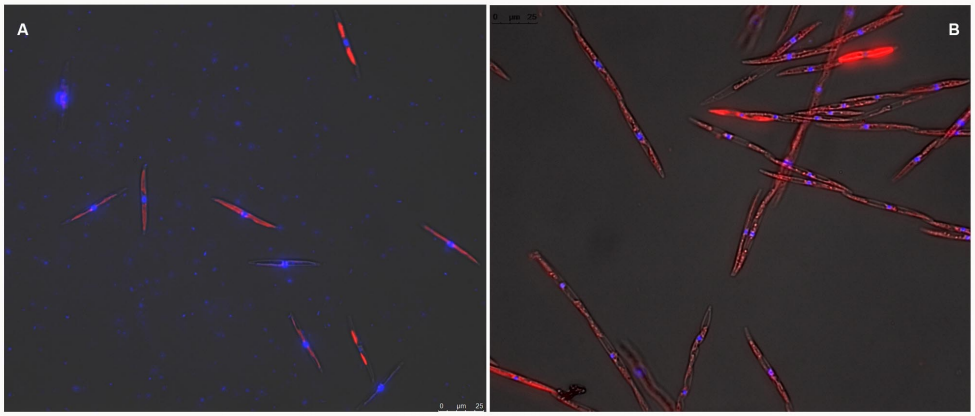
3 Transfer the cultures into a variable higher volume flask containing F/2 medium and antibiotics.
Arrive progressively at the final volume.

contamination test

4 Assess axenicity of the strains by fluorescence microscopy using DAPI staining (4',6-diamidino-2-phenylindole-a DNA stain).

(DAPI stains DNA, if bacteria are present in the culture bacterial nucleoids can be visualised as fluorescent spots).

To fix cells use neutralized formaldehyde 1.6%
DAPI final concentration 1:1000



Pseudo-nitzschia multistriata fluorescence microscopy images using DAPI staining.

A. Normal culture with bacteria
B. Axenic culture without bacteria.