Cryopreservation at the Roscoff Culture Collection

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The Roscoff Culture Collection (RCC: http://www.roscoff-culture-collection.org) currently holds over 6000 strains covering a broad range of the biodiversity of marine unicellular photosynthetic plankton (microalgae and cyanobacteria). The RCC also maintains bacteria, microalgal viruses and macroalgal strains. The main strategic focus of the RCC remains isolation, maintenance, characterization and distribution of strains.

Cryopreservation refers to the maintenance of biological samples in a state of suspended animation at low temperatures. The main aim is to preserve organisms without change in their morphological and genetic properties. Progressive cooling and use of cryoprotectants limit the formation of ice crystals inside the cells, limiting cell damage and therefore increasing cell viability upon thawing.

Two step cooling process

Step 1 : DMSO : 10 **% final**
Ramping : -1 °C/min
Start at 20 °C
→ *Using* ***Kryoplanner*** *360 3-3*

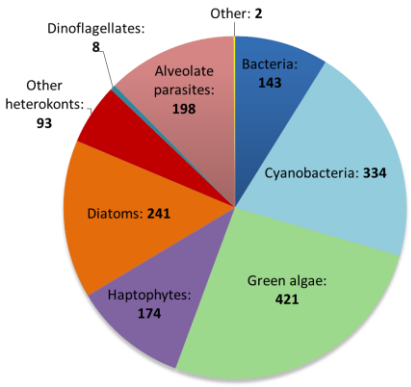
Step 2 : Cryotubes are plunged into liquid nitrogen or put in the -150°C freezer

Thawing process

- Plunge tubes for 3 mins in 27°C water bath
- Transfer contents of tube into flask with 20mL of culture medium
- Place at original culture temperature in dark
- After 24h reinstate original light conditions

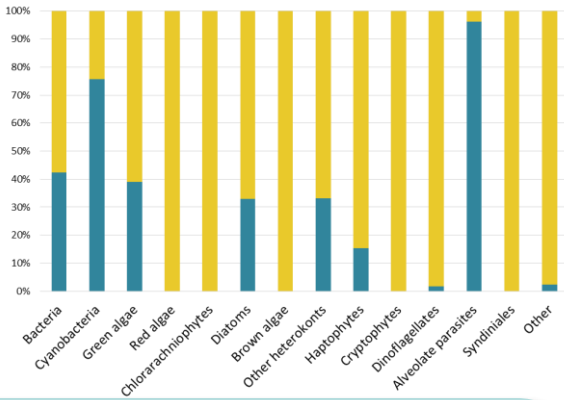
Strain viability after thawing is assessed by flow cytometry or microscopic observation

Diversity of cryopreserved strains at the RCC

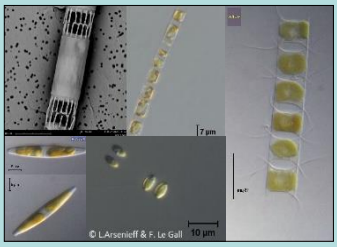


Over **1600 strains** cryopreserved

Percentage of cryopreserved strains by taxonomic groups

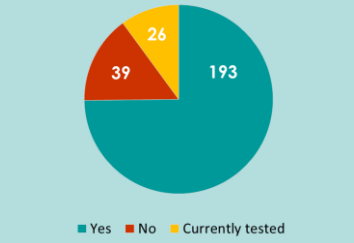


Cryopreservation of RCC diatom strains



The RCC holds over **700 strains** of diatoms including a large diversity of genera. Diatoms have regularly been reported to be difficult to successfully cryopreserve. At the RCC, cryopreservation has been tested on a total of **258 diatom strains** since October 2018. The majority of the strains tested belong to the genera *Nitzschia*, *Minutocellulus*, *Chaetoceros, Thalassiosira, Minidiscus,* and *Skeletonema.* Approximately **75%** of tested strains successfully grew after thawing.

Viability unfrozen of the diatoms cryopreserved strains 2018-2019



Perspectives

Cryopreserve 50 % of RCC strains

Continue routine cryopreservation of cryo-amenable species, such as green algae, haptophytes and diatoms.

Provide a cryopreservation service for the scientific community

Trials on untested groups and development of alternative methods for cryo-recalcitrant groups

- Macroalgal gametophytes
- Cryptophytes
- Chlorarachniophytes
- Dinoflagellates (*Alexandrium*)

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