Available online at www.sciencedirect.com
SCIENCE DIRECT
CRYOBIOLOGY
Cryobiology 52 (2006) 152–156
www.elsevier.com/locate/ycryo

Brief communication

Cryopreservation of economically valuable marine micro-algae in the classes Bacillariophyceae, Chlorophyceae, Cyanophyceae, Dinophyceae, Haptophyceae, Prasinophyceae, and Rhodophyceae ☆

Lesley Rhodes [a,*], John Smith [b], Robin Tervit [b], Rodney Roberts [a], Janet Adamson [a], Serean Adams [a], Marie Decker [c]

[a] *Cawthron Institute, Private Bag 2, Nelson, New Zealand*
[b] *AgResearch, Ruakura Agricultural Research Centre, PO Box 3123, East Street, Hamilton, New Zealand*
[c] *ENSIL, 16, rue Atlantis, 87068 LIMOGES, France*

Received 13 May 2005; accepted 7 October 2005
Available online 29 November 2005

Abstract

The ability to routinely cryopreserve micro-algal species reduces costs associated with maintaining large culture collections and reduces the risks of losing particular strains or species through contamination and genetic drift. Cryopreservation is also a useful adjunct in aquaculture hatcheries for strains of micro-algae where the nutritional status may change as a result of continuous sub-culture. In this study, cryopreservation of isolates from seven micro-algal classes was investigated. Successful candidates included the marine dinoflagellates *Amphidinium carterae*, *Amphidinium trulla*, and *Gymnodinium simplex*, and the haptophytes *Chrysochromulina simplex*, *Prymnesium parvum*, *Prymnesium parvum* f. *patelliferum*, *Isochrysis galbana*, and *Pavlova lutheri*. Also successfully cryopreserved were the planktonic diatoms *Chaetoceros calcitrans*, *Chaetoceros muelleri*, *Chaetoceros* sp., and the benthic *Nitzschia ovalis*, the chlorophyte *Chlamydomonas coccoides*, the rhodophyte *Porphyridium purpureum*, the prasinophytes *Tetraselmis chuii*, and *Tetraselmis suecica*, and the cyanophytes *Raphidiopsis* sp., and *Aphanizomenon flos-aquae*. All species were successfully cryopreserved using 15% Me_2SO.

Keywords: Cryopreservation; Micro-algae; Dinoflagellates; Bacillariophyceae; Chlorophyceae; Cyanophyceae; Dinophyceae; Haptophyceae; Prasinophyceae; Rhodophyceae; Dimethyl sulfoxide (Me_2SO)

Marine micro-algae represent a rich and diverse genetic resource and interest in their biotechnology potential is increasing rapidly. This interest is expressed in the collections of potentially economically valuable marine micro-algae maintained around the world [2,9,7]. The collection of toxic micro-algae is critical for a variety of applied and

☆ The work was funded by the NZ Foundation for Research Science and Technology (contract CAWX0304).
* Corresponding author. Fax: +64 3 546 9464.
E-mail address: Lesley.rhodes@cawthron.org.nz (L. Rhodes).

0011-2240/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cryobiol.2005.10.003

fundamental research uses, including phytoplankton monitoring, development and testing of DNA probes, and mass production of bioactive compounds with pharmaceutical potential, for example neuroprotective agents. Mass production of these compounds is required for chemical characterization, toxicological studies and the preparation of biotoxin standards. However, the continuous maintenance of large culture collections is costly and there is a constant risk of strains or species becoming contaminated, and of genetic drift occurring with serial sub-culturing. The ability to routinely cryopreserve micro-algae would circumvent some of these issues and enable a larger number of strains to be maintained.

Micro-algae are also cultured in aquaculture hatcheries as food for juveniles. These species, mainly haptophytes and diatoms, have been selected for their nutritional value, ease of culture, and proven performance. Cell size, culture vigour, and nutritional value may change over time in culture and preservation of these isolates and strains is therefore desirable.

The Cawthron Institute maintains the nationally recognised micro-algae culture collection for New Zealand, the Cawthron Culture Collection of Micro-algae, which contains a number of species that are endemic to New Zealand waters. The collection also maintains several species that are of interest for their bioactive compounds, as well as those that are used in New Zealand aquaculture hatcheries. A long-term goal is to cryopreserve most of the species in the collection for the reasons outlined above.

This study reports the successful cryopreservation of 23 micro-algal isolates (representing 18 species from 7 classes) and includes the first report of the cyropreservation of photosynthetic marine dinoflagellate species (Table 1).

The cultures of micro-algae selected to be assessed for cyropreservation success were maintained in the Cawthron Culture Collection. They were grown to stationary phase in standard growth media [11,6] under standard growth conditions of 100 μEin m^{-2} s^{-1} light intensity and 19 °C (Table 1).

Thirty-nine micro-algal strains, representing 33 species (Table 1) were screened for cyropreservation success using the following standard protocol. Micro-algae were added to 5 mL glass culture tubes (Kimble Glass, Vineland, NJ) in 1 mL aliquots and diluted 1:1, at room temperature (≈20 °C), with the permeating CPA dimethyl sulfoxide (Me_2SO) to give final concentrations of 10 and/or 15% (v/v). The CPA solutions (at double the final concentration desired, and in the preferred growth medium for each species) were added in steps of 100 μL each min for 10 min with gentle agitation after each addition. After the final addition, the tubes were stoppered and left to equilibrate for 30 min, approximately 15 min of which occurred in the dark. Each treatment was carried out in triplicate.

After approximately 15 min in the dark, algae were aspirated into cryopreservation straws (0.5 cc, IMV, France), plugged with coloured PVC powder, and placed in water to set the powder. Straws were then wiped dry and held horizontally on a rack until the 30 min equilibration time had been reached. They were then transferred to controlled-rate freezers (Cryologic Pty, Mt Waverley, Australia) programmed to cool from 20 to −40 °C at 3 °C min^{-1}. Straws were then held at −40 °C for 10 min before being plunged into liquid nitrogen (−196 °C).

Straws were thawed by removing from liquid nitrogen and plunging into a water bath at ≈20 °C. The straw contents were then individually diluted by step-wise addition of 500 μL of their standard growth medium each minute for 10 min with regular gentle agitation between each addition. After equilibration in the dark for 30 min, a final 5 mL of standard growth medium was added. Micro-algae were on-grown in 50 mL bottles (Labserv, Biolab, Auckland, New Zealand) for 24–48 h in the dark, a further 48–72 h under red light (tube reference: OSRAM L18W/60, Germany), and then under the standard light conditions outlined above. A control straw from each treatment was processed as above but not cryopreserved to test the effect of CPA exposure and handling on algae survival independent of the freezing process.

Twenty-two isolates were successfully cryopreserved using the standard protocol (Table 1). *Tetraselmis chuii* and *Tetraselmis suecica* were initially included as positive controls, as published protocols are available for them [4,3]. *Isochrysis galbana*, *Chlamydomonas coccoides*, and *Chaetoceros calcitrans* have also been previously cryopreserved [13,10].

The small haptophytes, *Chrysochromulina simplex* (≈8 μm diameter), *Prymnesium parvum*, and *Prymnesium parvum* f. *patelliferum*, survived cryopreservation with 15% Me_2SO as did three dinoflagellates, *Amphidinium carterae*, *Amphidinium trulla*, and *Gymnodinium simplex* (Table 1). These three latter species are small in comparison to *Togula*