

Cryopreservation studies on the marine diatom *Navicula subinflata* Grun

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ABSTRACT

Very little work has been done on marine unicellular algae regarding cryopreservation. The present work was, therefore, undertaken to study the effect of different cryoprotectants and cryopreservation on the growth of marine diatom *Navicula subinflata*. The effect of cryoprotectants such as Ethylene glycol, DMSO, Methanol, Glycerol and Sucrose was studied on *Navicula subinflata* at 1M, 2M, 4M and 6M concentrations for a period of 30 and 60 minutes to find out the optimum concentration for the growth of diatom for cryopreservation experiment. The optimum concentration of Ethylene glycol, DMSO and Methanol was found to be 4M in 30 minute experiment, while in 60 minute experiment no satisfactory growth was observed and increasing concentration of cryoprotectant decreased the growth. These experiments after cryopreservation revealed that the maximum growth was recorded in Ethylene glycol treated cells for 0 day sample and 7 days and 30 days liquid nitrogen preserved samples. No growth was observed in Methanol (4M) treated cells for 7 days, 15 and 30 days liquid nitrogen preserved samples. Thus the present work reveals the possibility of application of cryopreservation in marine algae.

Introduction

Freezing is known to be lethal to most living systems yet it can also preserve cells and their constituents. It can slow or stop some bio-chemical reactions within cells. Supercooling is used to preserve a fine structure of cells. Cryopreservation studies have been made by Fenwick and Day (1992) on *Tetraselmis suecica*, Arbault and Delanoue

(1994) on *Porphyra linearis* and Conavate and Lubian (1994 and 1995) on marine microalgae.

The present work deals with the cryopreservation of the marine diatom *Navicula subinflata* and its response to growth after keeping in liquid nitrogen (-196°C) at varying periods. The result is a step in understanding the requirement for a diatom seed bank in aquaculture since diatoms contribute

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as an important food resource especially in shellfish hatchery.

Material and Methods

Cells from the exponential phase of *Navicula subinflata* culture were taken for the experiment. 10 ml of culture was centrifuged and the suspension was mixed in cryoprotectants such as Ethylene glycol, Dimethyl sulphoxide (DMSO), Glycerol and Sucrose at varying concentrations, from 1M to 6M and the cells were treated for a period of 30 minutes and 60 minutes. The optimum concentration of cryoprotectants observed in these experiments was taken for further cryopreservation experiment. The cells were treated in 3 cryoprotectant media (Ethylene glycol, DMSO and Methanol) at 4M concentration and these treated cells were cryopreserved using Kryo-10 instrument. The cooling rate was 1°C/minute. After cooling the cells up to -40°C, the cells were taken out and were kept in liquid nitrogen for a period of 7, 15 and 30 days. One set of experiment was directly taken for the growth studies (zero day) and remaining 3 sets were taken out of liquid nitrogen after 7, 15 and 30 days and the cells were placed in 10ml F/2 medium for further growth studies.

Results

The effect of cryoprotectants such as Ethylene glycol, DMSO, Methanol, Glycerol and Sucrose was studied on *Navicula subinflata* in 1, 2, 4 and 6M concentrations for the period of 30 minutes and 60 minutes to find out the optimum concentration for the growth of diatom for cryopreservation experiment and the results are presented in Figure 1 to 6. The optimum concentrations of Ethylene glycol, DMSO and Methanol were found to be 4M in 30 minutes experiment, while in 60 minutes experiments no satisfactory growth was observed and increasing concentration of cryoprotectants

decreased the growth of diatom. Also, maximum growth was recorded in Ethylene glycol treated cells for 0, 7 and 30 days liquid nitrogen preserved samples, while no growth was observed in Methanol (4M) treated cells for 7, 15 and 30 days in similar samples.

Discussion

Though reports are available on cryopreservation effect on marine algae (Fenwick and Day, 1992; Arbault and Delanoue, 1994; Conavate and Lubian, 1994 and 1995), work on marine diatoms is very scanty. It deals with only tolerance of *Chaetoceros* to DMSO and Methanol. Thus the present results are compared only with those of Conavate and Lubian (1994) on *Chaetoceros gracilis*. However, regarding growth of diatoms after keeping in liquid nitrogen, no information is available. Conavate and Lubian (1994) studied tolerance of *Chaetoceros gracilis* to DMSO and Methanol and observed that it completely lost viability when exposed to 20% concentration of DMSO for 60 minutes. Lower concentrations for DMSO incubations were similar (5% lower) to lethal thresholds. Methanol incubation did not significantly decrease cell viability at concentrations of 15% up to 20 minutes but it was observed to be dependent on 15% DMSO preincubation to achieve a growth response after thawing from -196°C.

In the present study, the optimum concentration of DMSO and Methanol for *Navicula subinflata* was found to be 4M for 30 minutes. The 4M concentration of DMSO was not significant for this species, for 60 minutes treatment but with Methanol the same concentration was found to be lethal. The growth experiments after cryopreservation revealed that maximum growth was recorded in Ethylene glycol (4M) treated cells for 0, 7 and 30 days liquid nitrogen preserved samples, while the 15 days liquid nitrogen

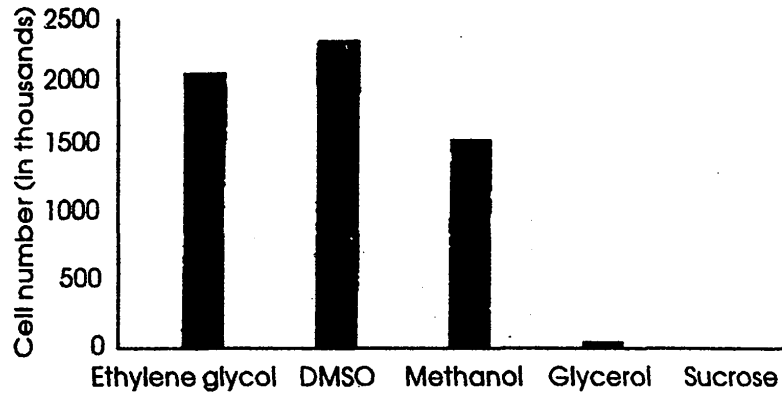


Fig. 1. Growth of *Navicula subinflata* after treatment in various cryoprotectants in 30 minutes period at 4 M concentration

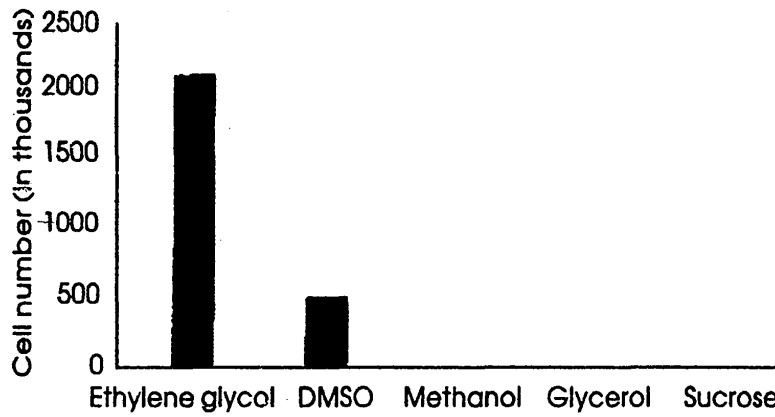


Fig. 2. Growth of *Navicula subinflata* after treatment in various cryoprotectants in 60 minutes period at 4 M concentration

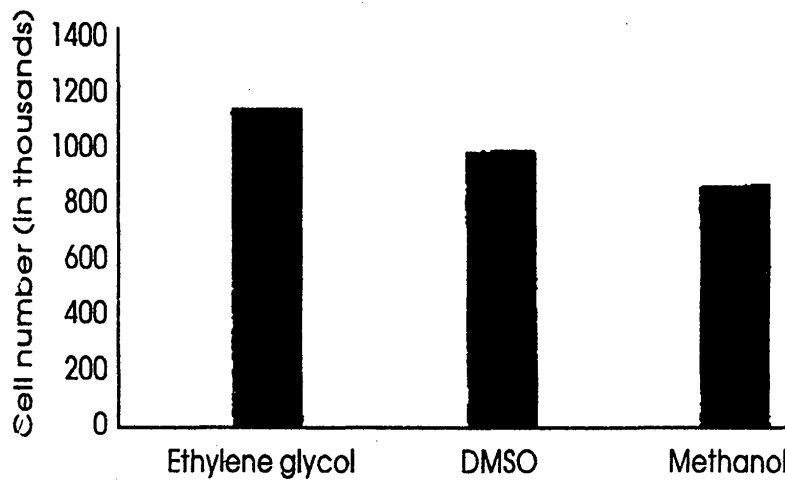


Fig. 3. Growth of *Navicula subinflata* after keeping in liquid Nitrogen for 0 day

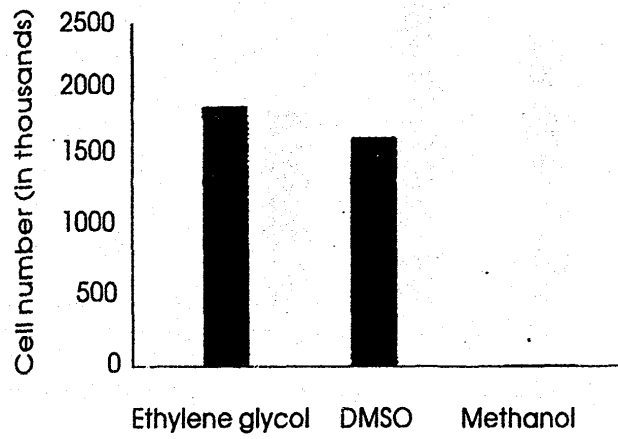


Fig. 4. Growth of *Navicula subinflata* after keeping in liquid Nitrogen for 7 days

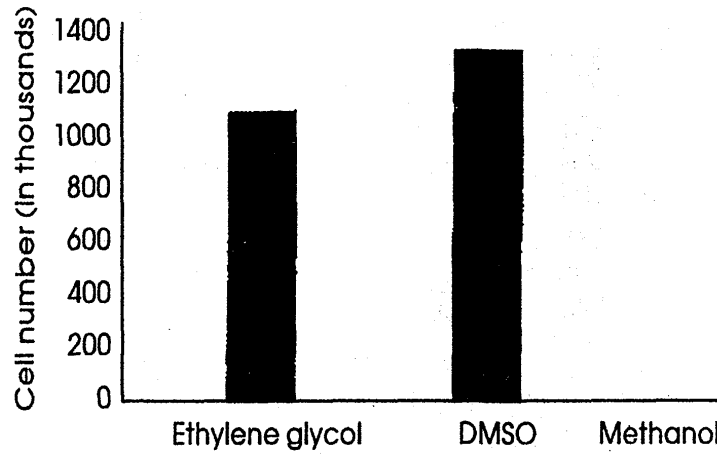


Fig. 5. Growth of *Navicula subinflata* after keeping in liquid Nitrogen for 15 days

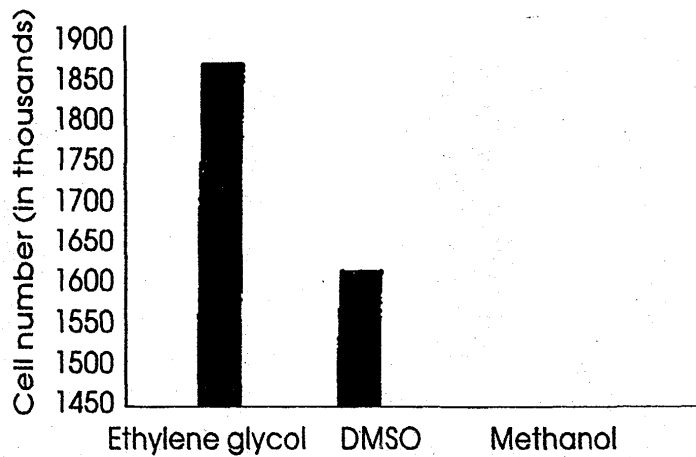


Fig. 6. Growth of *Navicula subinflata* after keeping in liquid Nitrogen for 30 days

preserved sample showed maximum growth in DMSO (4M) treated cells. Minimum growth was recorded in Methanol (4M) treated cells for 0 day liquid nitrogen preserved sample, while 7, 15 and 30 days samples showed no growth.

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