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METHODOLOGICAL STUDY OF THE CRYOCONSERVATION PROCESSES IN MICROALGAE

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Curso 2014/2015

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Trabajo Fin de Título para la obtención del título de Grado en Ciencias del Mar

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Abstract

Two cryopreservation methods, controlled cooling and encapsulation/vitrification, were studied in order to find an appropriate protocol to maintain microalgae cultures by exposing them to ultra-low temperatures (cryogenics). This study has shown that the most efficient cryopreserving method is the use of cryoprotectants, being Glycerol and DMSO the best options for this procedure, and dismissing the encapsulation/vitrification method due to the low effectiveness, which results in a low post-thaw viability rate and a higher demanding of labour and consumables.

Key words: biodiversity, microalgae, cyanobacteria, cryopreservation, cryoprotectants, conservation

Resumen

Dos métodos de criopreservación, enfriamiento controlado y encapsulación/vitrificación, han sido estudiados con el objetivo de encontrar un protocolo adecuado para conservar los cultivos de microalgas mediante su exposición a muy bajas temperaturas (criogenización). Este estudio ha demostrado que el método de criopreservación más eficiente es el de enfriamiento controlado combinado con el empleo de crioprotectores, siendo el Glicerol y DMSO los crioprotectores más adecuados para este procedimiento. A su vez se descarta el método de encapsulación/vitrificación debido a su baja efectividad, que se traduce en una baja tasa de viabilidad tras el proceso de descongelación y supone un método con una mayor demanda de tiempo de trabajo y de material.

Palabras clave: biodiversidad, microalgas, cianobacteria, criopreservación, crioprotectores, conservación

Introduction:

Microalgae have proved to be a key player in the biotechnology field. This organisms produce a wide variety of important bioactive compounds, which can include

antitumor (13 %), antiviral (4 %) and antibiotics (12 %) among many others in case of cyanobacteria (Rastoll et al., 2013).

Nowadays, there are numerous microalgae collections that provide cultures for different fields and disciplines as biotechnology, medicine, gastronomy, education, etc. This collections maintain their strains in stable state generally by serial sub-culturing to fresh medium after a certain time of growth, but this method has disadvantages like being labour-intensive and especially susceptible to cross-contamination during handling.

For that reasons, cryopreservation techniques have been explored in recent years. This technique seems to be a crucial part of the storage and biodiversity conservation in many fields, like the biotechnology. By means of cryopreserving important and valuable microalgae cultures we can ensure the long-term storage of the strains and reduce not only process variability from genetic rift but the need to maintenance and handling, reducing the risk of possible culture contamination. Cryopreservation, also permits collections to expand avoiding the costs associated with the maintenance of the collection, enough reasons to be the method of choice for many collections to conserve and maintain important organisms.

The current goal in this study is to provide valuable information about the optimal growth state in which it is correct to start cryopreserving the cells and to find evidences that prove the success on different cryopreservation processes and the viability of the strains once the cultures return from the freeze state into their original state, after the thawing procedure.

Material and methods:

Culture and maintenance

The different strains used in this study (Fig. 1) were mainly isolated during programmed bioprospecting in different environments (mostly in Canaries regions). A total of 4 strains were used in this study which can be grouped depending on the origin ecosystem: fresh waters and marine waters. All strains are deposited in the Spanish Bank of Algae.

All the studied strains were cultured without agitation in Erlenmeyer flasks with appropriate culture medium and placed in different growth chambers under different illumination provided by cool white fluorescent lamps, with determined photoperiod cycle (Table 1).

Table 1: Code and strain information

Code	Strain	Division	Type	Culture médium	Geographical location	Temperature	Light
BEA 0398	*Picochlorum oklahomense*	Chlorophyte	Hypersaline	F/2	Spain, Canary Islands, Fuerteventura. Saltworks del Carmen.	23°C	168 50 µmol photones·m^{-2}· s^{-2}. 18:6 (L:D) photoperiod
BEA 0544B	*Coccomyxa sp.*	Chlorophyte	Fresh water	BBM+vit.	Spain, Canary Islands, La Gomera. Garajonay National Park. Little stream.	23°C	168 50 µmol photones·m^{-2}· s^{-2}. 18:6 (L:D) photoperiod
REC 0017B	*Synechococcus sp.*[+]	Cyanobacteria	Fresh water	BG11	Spain, Zaragoza. La Tranquera reservoir	23°C	50 µmol photones·m^{-2}· s^{-1}. 14:10 (L:D) photoperiod
REC 0057	*Synechococcus sp.*[+]	Cyanobacteria	Brackish water	BG11	Poland, Gulf of Gdansk, Baltic Sea.	23°C	168 50 µmol photones·m^{-2}· s^{-2}. 18:6 (L:D) photoperiod

[+]Antibiotic capacity found

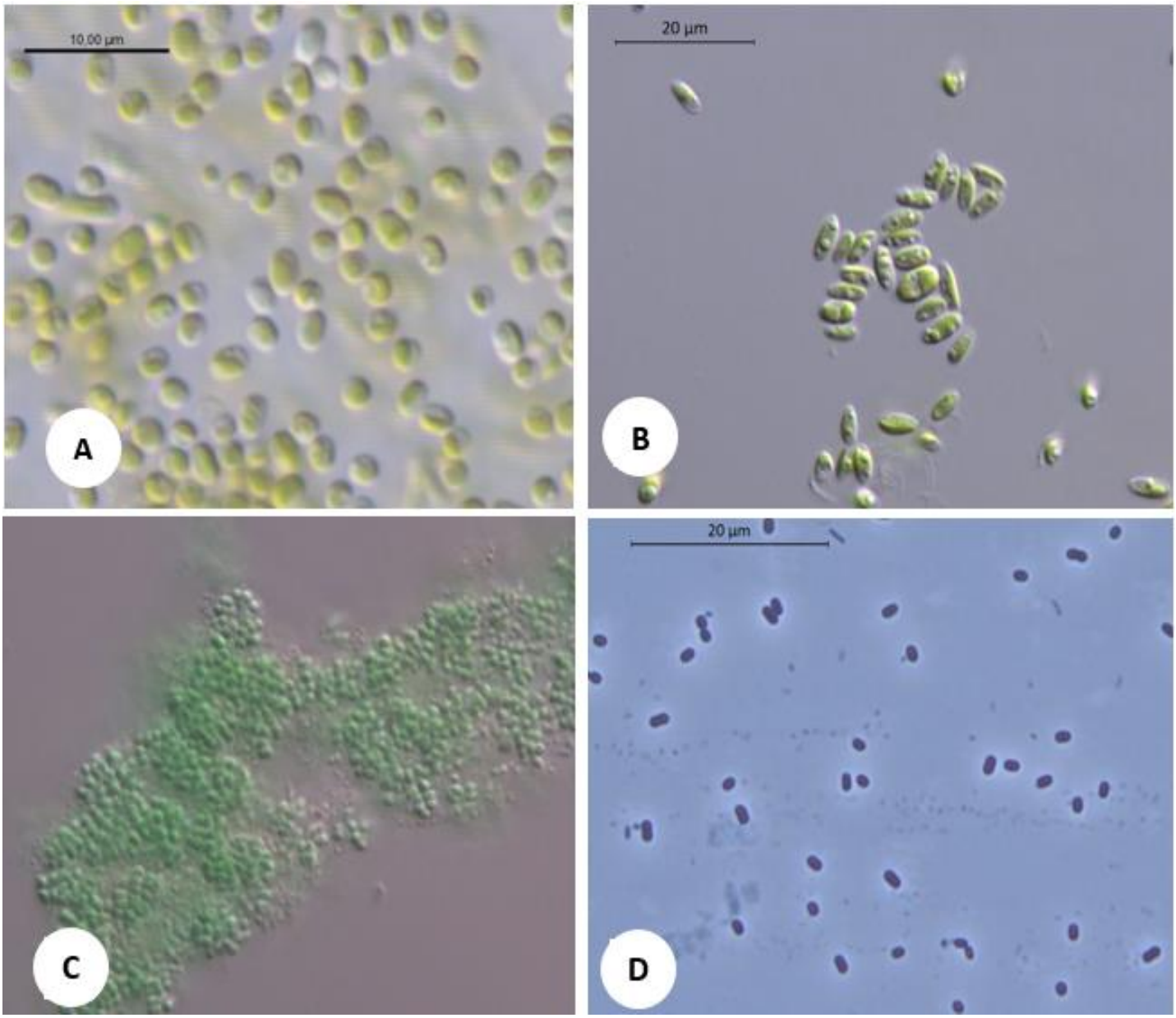

Fig. 1: All tested strains: (a) BEA 0398 *Picochlorum oklahomense*. (b) BEA 0544 *Coccomyxa sp*. (c) REC 0017B *Synechococcus sp*. (d) REC 0057 *Synechococcus sp.*

Growth characterization

All cultures were cryopreserved using cells harvested from late exponential or early stationary growth phase with cell density ~ 2 x 10^7 (modified from Day et al., 2007) (Table 2), in order to ensure if this metabolic states were the optimal states in which it was suitable start the cryopreservation process. A growth curve was built for BEA 0398 *Picochlorum oklahomense*, BEA 0544B *Coccomyxa sp*. and REC 0057 *Synechococcus sp*.as control strains, using four different methods of measurement. Optical density was

measured spectrophotometrically (Perkin Elmer UV Lambda 25) (measuring in 665nm and 750nm) as well as Chlorophyll α that was also estimated spectrophotometrically (measured in 665.2nm, 652.4nm and 470nm) after pigment extraction with methanol. Dry weight was estimated by filtering the sample in a known weighed filter (0.2µm pore size) and number of cells estimated by flow-cytometry (Cell Lab Quanta SC). REC 0017B *Synechococcus sp.* growth curve wasn't built due to technical issues.

Table 2: Cultures cell density in the moment of the cryopreservation process estimated by flow-cytometry

Strain	Treatment	Growth state	Cell/ml
BEA 0398 Picochlorum oklahomense	Cryoprotectant*	Stationary	$2{,}2 \cdot 10^7$
	Encapsulation-dehydration	Stationary	$2{,}2 \cdot 10^7$
BEA 0544B Coccomyxa sp.	Cryoprotectant*	Stationary	$2 \cdot 10^7$
	Encapsulation-dehydration	Stationary	$2{,}7 \cdot 10^7$
REC 0017B Synechococcus sp.	Cryoprotectant*	Exponential	$2 \cdot 10^5$
	Encapsulation-dehydration	Exponential	$2{,}2 \cdot 10^5$
REC 0057 Synechococcus sp.	Cryoprotectant*	Late exponential	$9{,}6 \cdot 10^7$
	Encapsulation-dehydration	Late exponential	$2{,}13 \cdot 10^7$

*Treated with DMSO, MeOH and Glycerol.

Experimental design

Two different common cryopreservation approaches were employed; (a) controlled cooling and (b) encapsulation/vitrification, allowing the testing of different critical point cryogenic factors and cryoprotective approaches. The methods employed included the use of traditional chemical cryoprotectants followed by exposure to a low temperature gradient employing a Mr. Frosty®, a passive cooling chamber. This vessel is placed in a -80ºC freezer (Day et al., 2007) prior to transferring the cryovials to an ultra-freezer (-150º C). Three different cryoprotectants were tested: Glycerol, Dimethyl sulphoxide (DMSO) and Methanol (MeOH). The encapsulation method involved alginate encapsulation. Vitrification method included encapsulation followed by a cryoprotective osmotic process using a glucose gradient and evaporative dehydration. These treatments increase cell viscosity to a critical point at which water forms an amorphous metastable,

glassy state on exposure to ultra-low temperatures (modified from Day et al., 2007). (Fig. 3).

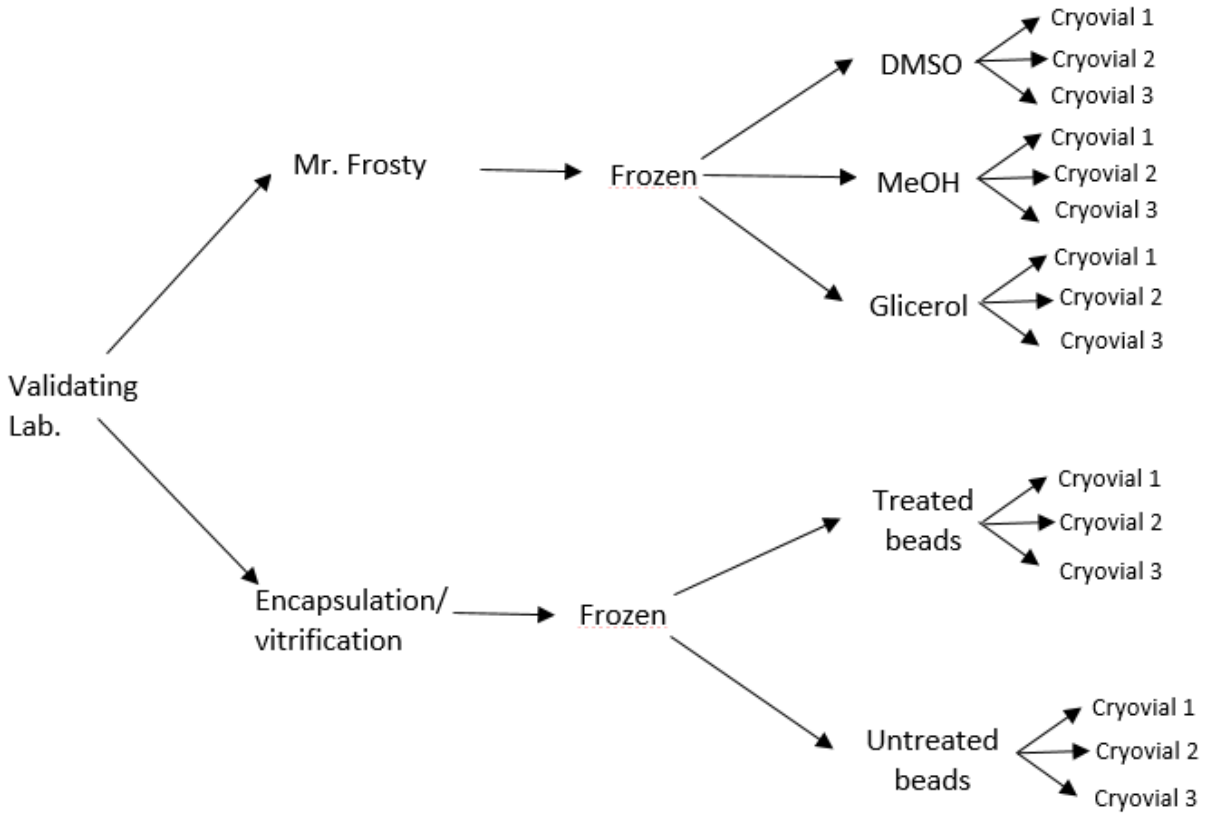

Fig. 3: Experimental design applied to samples taken from clonal cultures; separated in three replicated cryovials: (a) controlled cooling with three traditional chemical cryoprotectants. (b) encapsulation/vitrification. Treated beads were encapsulated followed by a cryoprotective osmotic process and evaporative dehydration. Untreated beads were encapsulated in alginate and frozen immediately after bypassing the dehydration process.

Controlled rate freezing protocol (Mr. Frosty)

Three different cryoprotectant solutions were prepared with appropriate culture media for each strain. 10 ml of culture media and cryoprotectant DMSO, Glycerol and MeOH with a concentration of 10% (v/v), were prepared in order to start the cryopreservation process. Aliquots (0.9 ml) of each strain were transferred to three 2 ml cryovials with 0.9 ml of cryoprotectant solution DMSO, Glycerol and MeOH, respectively, resulting in a final concentration of 5% (v/v). The tubes were mixed in order to ensure the good cryoprotectant penetration in cells and left in darkness for 40 minutes. After the compensation time, cryoprotected cells where then transferred to the Mr. Frosty, set up in accordance with the manufacturers' instructions: Mr. Frosty unit was filled with

isopropanol (C_3H_8O – IPA) to the 250 ml graduation mark and placed in a refrigerator at 4°C previous to the use. The Mr. Frosty with the cryoprotected cultures content in 2 ml cryovials was placed in a -80°C freezer and incubated for 1.5h, which gave a cooling rate of -1°C/min. After 1.5h cryovials were removed from Mr. Frosty and transferred to -150°C ultrafreezer ending the process (Day et al., 2007). This procedure was repeated for each triplicate strain, resulting in 9 frozen samples of each one.

Encapsulation/vitrification protocol

Aliquots (10ml) of each culture were took and a solution of alginate and distilled water was prepared (300 mg alginate for each 10 ml water). The cultures were then mixed in a proportion 1:1 and slowly dispensed drop wise using a syringe into 150 ml of sterile 0.1M $CaCl_2$ solution. The formed beads were equilibrated into de $CaCl_2$ solution for 60 minutes and then removed using a sterile filter. Part of the untreated beads where isolated and cryopreserved, stored in -150°C ultrafreezer into 2ml cryovials (10 beads per cryovial). The rest of the beads were transferred into a first osmotic dehydration medium (sterile culture medium and 0.5M sucrose) where they were incubated for 24h. under standard conditions of light and temperature. After 24h. the 0.5M sucrose medium was decanted and replaced with another osmotic dehydration medium (sterile culture media and 0.75M sucrose) and the beads were incubated another 24h. under the same conditions. Once the vitrification process was finished, the beads were retrieved from the solution by blotting the sucrose medium on sterile filter. The beads were placed then in sterile 9cm Petri dishes, ensuring the beads were not in physical contact with each other, and incubated in a horizontal laminar air-flow for 3 h. Once the beads were desiccated, were transferred into 2ml cryovials and stored in -150°C ultrafreezer (Day et al., 2007). A minimum 60 beads of each culture were stored in 6 different cryovials (10 per cryovial); 3 cryovials with untreated beads and the other 3 cryovials with dehydrated beads.

Thawing following controlled rate freezing protocol

Stored cryovials were thawed in a pre-headed water bath (37°C) for 3 min. until all the ice had melted and left in darkness for an interval of 24h. The samples were then centrifuged (1000 x g 10 min.) the supernatant discarded and the pellet re-suspended in 7

ml fresh culture medium in order to remove the cryoprotectant and avoid possible damages to the cells due to the chemical compound (modified from Rastoll et al., 2013; Day and Brand, 2005). Vital staining was performed using fluorescein diacetate (FDA) for viability assays using flow-cytometry.

Thawing following encapsulation-dehydration vitrification protocol

Stored cryovials were thawed as described above. After the thaw the non-dehydrated beads were left in darkness during 24 h. in 1 ml of fresh culture medium. Dehydrated beads were transferred to 5 ml of appropriate growth media for 1 h. re-hydration. After 1 h. the beads re-hydrated were placed in a Petri dish containing 1 ml of fresh culture medium and left for 24 h in darkness. After the equilibration time 5 beads were placed in 1.5 ml of culture media containing 3% (w/v) sodium hexametaphosphate ($Na_6P_6O_{18}$) and left for 40 min. to dissolve the alginate and release the cells, which were then centrifuged (1000 x g for 10 min) the supernatant discarded to remove residual $Na_6P_6O_{18}$ and the pellet re-suspended in 7 ml of fresh culture medium (modified from Day et al., 2007). Vital staining also was performed using FDA for viability assays.

Table 3: encapsulation protocol timetable. Expressed in days.

Task following encapsulation protocol	Days				
	I	II	III	IV	V
A. Cryopresrvation protocol					
A.1. $CaCl_2$ solution preparation					
A.2. Alginate solution preparation					
A.3. Alginate beads formation					
A.4. Beads freezing					
B. Thawing protocol					
B.1. Beads thawing					
B.2. Beads hydration					
B.3. Decapsulation					
B.4. Vital staining/viability assay					

Table 4: encapsulation/vitrification protocol timetable. Expressed in days. A longer process compared to just encapsulation procedure.

Task following encapsulation-dehydration protocol	Days				
	I	II	III	IV	V
A. Cryopresrvation protocol					
A.1. $CaCl_2$ solution preparation					
A.2. Alginate solution preparation					
A.3. Alginate beads formation					
A.4. First dehydration process					
A.5. Second dehydration process					
A.6. Beads freezing					
B. Thawing protocol					
B.1. Beads thawing					
B.2. Beads hydration					
B.3. Beads re-hydration					
B.4. Decapsulation					
B.5. Vital staining/viability assay					

Viability Assays

Viability assays of cells after cryopreservation was estimated by flow-cytometry (Cell Lab Quanta SC) after vital staining using fluorescein diacetate (FDA).

For the staining using FDA coloring solution, this chemical compound was stored in acetone at -20°C at a stock concentration of 5 mg ml^{-1} and added to the cultures samples in a concentration of 5µl ml^{-1}. The samples were then incubated for no more than 30 min. before processing with the flow-cytometer (Cell Lab Quanta SC) (modified from Fleck et al., 2006). In this case FDA is absorbed in viable cells, resulting in a cell fluorescence increase.

Viability was expressed as a percentage ratio of the number of viable cells to the total cell number in the cryopreserved cultures (Day et al., 2007).

% viability = (Nº post-treatment FDA positive) / (Total nº observed in field of view) x 100

Results:

Cultures viability after thawing in terms of cell density, are shown in Table 5. Cultures harvested from late exponential (REC 0057 *Synechococcus sp.*) and stationary growth phases (BEA 0544B *Coccomyxa sp.* and BEA 0398 *Picochlorum oklahomense*)

(Fig. 2), presented higher cell density than the culture harvested from exponential growth state (REC 0017B *Synechococcus sp*.) (Table 2). Nevertheless, all cultures, regardless of the growth phase, presented a high viability rate with cryoprotectant treatment (Table 5, Fig. 6.a.).

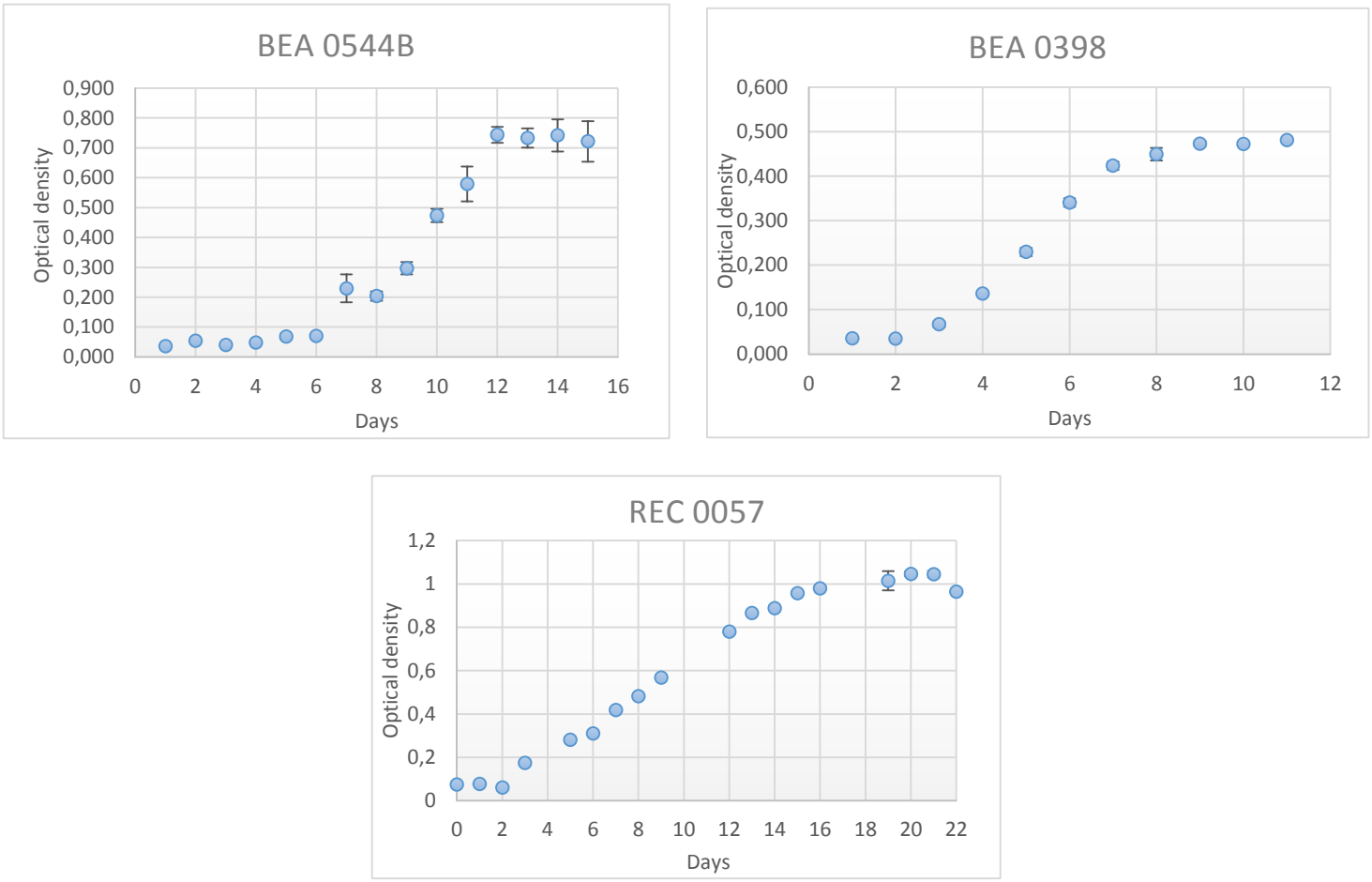

Fig. 2: Growth curves of three of the four tested strains. BEA 0398 (*Picochlorum oklahomense*), who presented the faster growth period, and BEA 0544B (*Coccomyxa sp*.) were harvested at stationary growth phase. REC 0057 *Synechococcus sp.* was harvested at late exponential growth phase.

All cryopreserved cultures viability were influenced by both controlled rate freezing and encapsulation/dehydration treatments. The methodology used in this study based on vital staining showed that cryoprotectant treatment was more effective than encapsulation/dehydration. Over the 4 tested strains, 3 of them presented higher viability when treated with cryoprotectant rather than encapsulation. DMSO and Glycerol were shown to be better cryoprotectants than MeOH (Table 5, Fig. 5 and 6.a).

The efficiency of cryoprotectants method was also observed in all the cultures after 2 days post-thawing recovery, resulting in a progressive increase coloration of the cells (Fig. 4.a)

Table 5: Viable cell number according to FDA signal measured in the flow-cytometer. Viability levels (%) using different treatments (DMSO, MeOH, Glycerol, encapsulation, encapsulation/vitrification).

Strain	Treatment	N[1]	Total cell/ml (x10^7)	Viable cell/ml (x10^7)	Viability (%)
BEA 0398 ***Picochlorum oklahomense***	MeOH	3	4,82	2,42	50
	DMSO	2	16,43	15,47	94
	Glicerol	2	17,63	16,24	92
	Encapsulation	1	0,043	0,03	70
	Encapsulation/vitrification	2	0,01	0,0035	33
BEA 0544B ***Coccomyxa sp.***	MeOH	2	0,87	0,28	32
	DMSO	2	8,8	1,69	19
	Glicerol	2	20,3	3,76	18,5
	Encapsulation	2	0,096	0,042	44
	Encapsulation/vitrification	2	0,014	0,003	20
REC 0017B ***Synechococcus sp.***	MeOH	3	29,75	12,66	42,5
	DMSO	2	80,1	64,6	80,7
	Glicerol	2	99,74	78,01	78
	Encapsulation	2	0,013	0,006	45
	Encapsulation/vitrification	2	0,33	0,034	10
REC 0057 ***Synechococcus sp.***	MeOH	3	19,97	6,69	33,5
	DMSO	2	41,98	11,87	28
	Glicerol	2	29,33	11,48	39
	Encapsulation	2	0,099	0,024	24
	Encapsulation/vitrification	2	0,1	0,03	0,5
Total		42			

[1]Number of replicates

Results in alginate encapsulation treatment showed that encapsulation without vitrification (non-dehydrated beads) was more effective than dehydration for all the tested strains (Table 5, Fig. 5). Moreover, the latter presented several difficulties when working with seawater strains hindering the formation of the alginate beads chemical structure (Fig. 4.b), which resulted in a more complicated and tedious protocol (Tables 3 and 4).

In general, during post-thawing, dehydrated capsules seem to be more resistant than non-dehydrated ones and present a better coloration (Fig. 4.c). Nevertheless, rehydration and decapsulation might be aggressive processes for the cells, given their low viability rate (Table 5, Fig. 5 and 6).

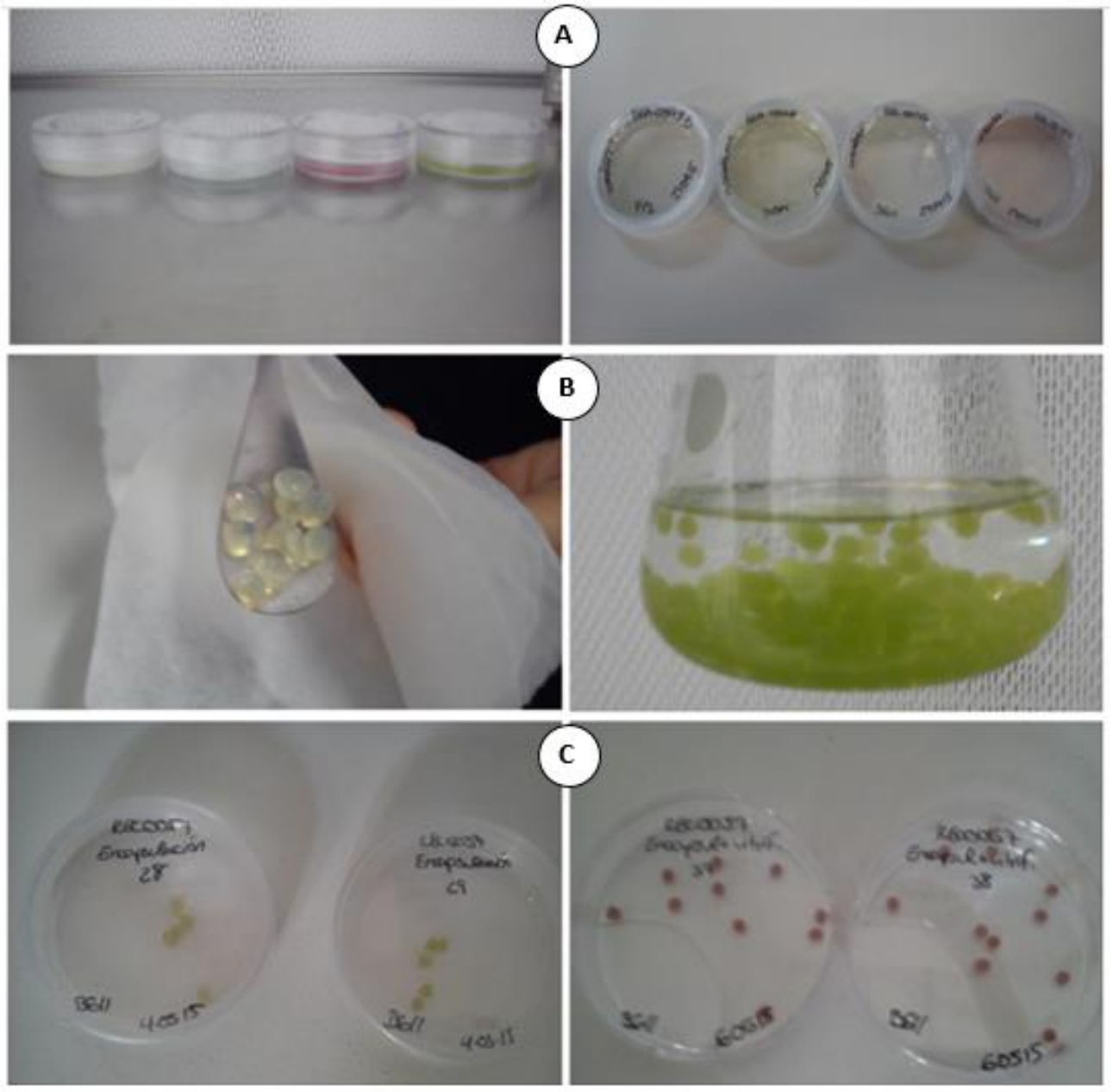

Fig. 4: (A) Post-thawing coloration evolution of the 4 cryopreserved strains, presenting a healthy appearance. (B) Left: BEA 0398 *Picochlorum oklahomense* encapsulation in seawater with the poorly-formed beads and low cell density. Right: BEA 0544B *Coccomyxa sp.* encapsulation in fresh water with well-formed beads and a good coloration, indicative of a good cell density in each bead. (C) Left: REC 0057 *Synechococcus sp.* encapsulation with an unusual yellowish color possibly due to the lack of nutrients inside the bead. Right: REC 0057 *Synechococcus sp.* encapsulation/dehydration with a bright red color and good appearance.

In general the highest viability in all the strains tested was observed with Glycerol and DMSO cryoprotectant treatments, with almost 60% viability rate both of them (Fig. 6.a), being BEA 0398 *Picochlorum oklahomense* the best candidate for cryopreservation with almost 70 % recovery (Table 5, fig. 6.b).

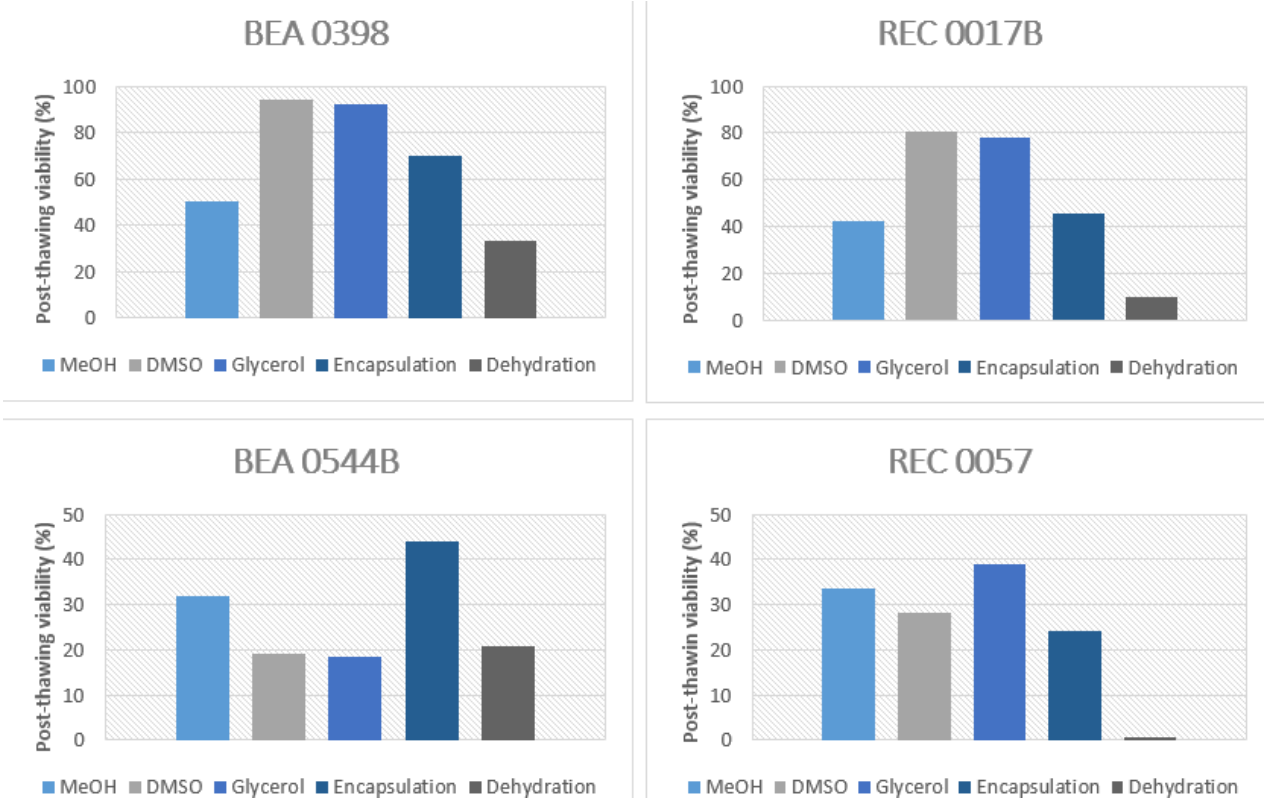

Fig. 5: Post-thawing recovery rate of four strains using different treatments.

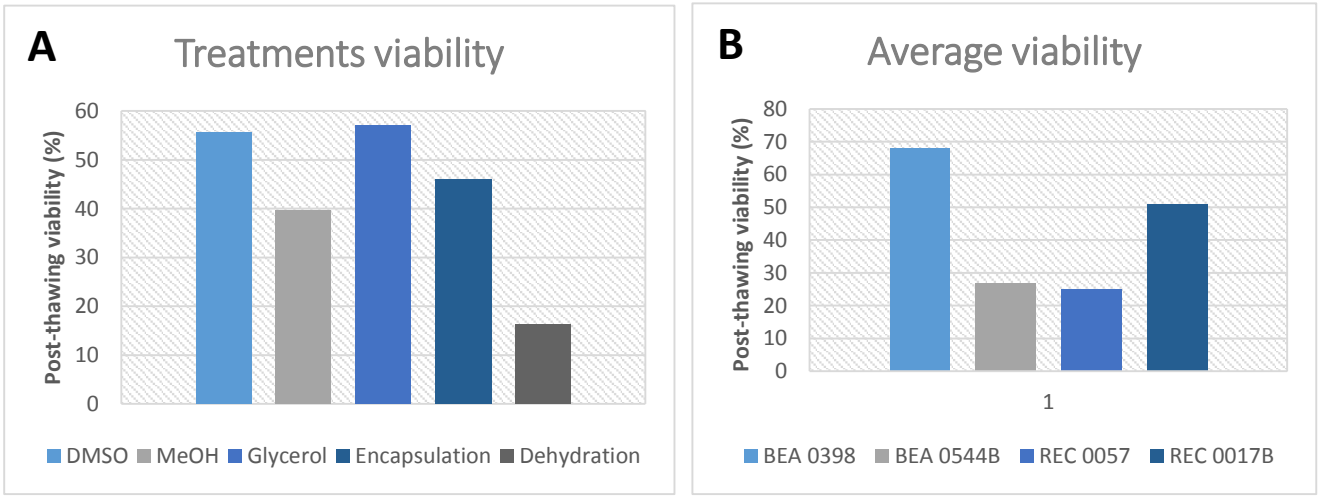

Fig. 6: A: Effectiveness of the different treatments applied to the cryopreserved cultures. B: Average viability of the 4 cryopreserved cultures.

Results in vital staining using FDA showed that the fluorescence signal measured with flow-cytometer was not influenced by the sample staining time, but by the cell wall structure. That explains the low viability rates in REC 0057 *Synechococcus sp.* and BEA 0544B *Coccomyxa sp.* (Table 5, fig. 5), whose thick cell walls hindered the penetration of the FDA into the cells.

Discussion:

The principle aims of this study were to: (I) test the effectiveness of two different cryopreservation methods (Controlled cooling and encapsulation/vitrification); (II) Evaluate the effects of three different traditional chemical cryoprotectants in the culture cells (DMSO, MeOH and Glycerol) followed by exposure to a low temperature gradient employing a Mr. Frosty® and (III) Make an evaluation of the accuracy of the different cryopreservation methods and assess their effectiveness in terms of time and economical spent needed to apply each protocol.

In this study we report a methodology for freezing, thawing and viability assessment for 4 different strains. Two different methods were tested: Controlled rate freezing with different cryoprotectants: DMSO, MeOH and Glycerol at a final concentration of 5% (v/v); and encapsulation/vitrification. Viability assessment applied consisted in vital staining with FDA.

Cryoprotectant function is to penetrate into the cells in order to facilitate the depression of the freezing point and reduce the excessive solute concentration to avoid intracellular ice nucleation and consequently cell damage and possible death (Fleck et al., 2006). Assays employing vital staining resulted in higher levels of post-thaw viability after cryoprotectant treatment compared to post-thaw viability after encapsulation/vitrification treatment (Table 5, fig. 6.a), DMSO being a better cryoprotectant than MeOH (Rastoll et al., 2013) (Fig. 5). Nevertheless results obtained in accordance with previous studies suggested that cryopreservation with Glycerol provides better viability rates, being the most efficient method compared with DMSO and MeOH (Esteves-Ferreira et al., 2012) as we demonstrate in this study (Table 5, fig. 6.a). Yet there was a strain, *Coccomyxa sp.* BEA 0544B, that preferred MeOH as cryoprotectant (Table 5, fig. 5).

These solvents are toxic for the cells and some strains are more susceptible to cell damage (Nunez-Vazquez et al., 2011; Urmeneta et al., 2003), but instead, there are other strains that presents a good resistance to this chemical compounds (e.g. BEA 0398 *Picochlorum oklahomense*) being the only saltwater strain tested and the one with the best viability rates (Table 5, fig. 6.b), similar results to those obtained in previous studies with saltwater cyanobacteria (Rastoll et al., 2013).

Low viability rates in REC 0057 *Synechococcus sp.* observed during vital staining assays may be due to FDA difficulty penetrating the thick cell-wall. However it was observed a good change in culture coloration after several days after being thawed (Fig. 4.a). This was considered as an evidence of good culture growth and physiological state, being a strain with a high recovery rate, as shown by previous studies (Rastoll et al., 2013)

In the case of encapsulation treatment this procedure presented several technical problems in beads formation, especially for marine strains, and in the assessment of algal viability and recovery, resulting in a low viability rate. For decapsulation procedure, alginate beads dissolution with $Na_6P_6O_{18}$ (Day et al., 2007) proved to be an inconvenient, especially for cyanobacteria REC 0057 and REC 0017B *Synechococcus sp*., due to the phycobiliprotein extraction in phosphate buffer during recovery processes. Hence this is an unsuitable method since it presents high cell-death rates, as previous studies have shown (Day et al., 2007).

Concluding, the results obtained prove that cells at stationary phase of the chlorophyte BEA 0544B *Coccomyxa sp.* isolated from fresh water environment showed high viability when encapsulation was used as cryopreservation method, proving to be a better procedure than encapsulation/vitrification, however the global low recovery rates and the time spent in the complex procedure, makes this technique a non-viable cryopreservation protocol (Tables 3 and 4). Otherwise, cells at exponential or stationary growth phase of planktonic coccoid cyanobacteria REC 0017B and REC 0054 *Synechococcus sp*. and chlorophyte BEA 0398 *Picochlorum oklahomense* shown better results when a control cooling method was applied using either DMSO or Glycerol as cryoprotectant. Also, in all four tested strains, the viability appeared to be unaffected by the cultures growth states, proving that is not necessary a determinate physiological state, but a good cell density, to start the cryopreservation process as suggested by previous studies (Day et al., 2007).

By last, it can be assumed that the viability data given by the flow-cytometer, depend on the characteristics of the cells. For that reason in some cases the measures and recovery rates may not be representative, as it is observed in REC 0057 *Synechococcus sp.* and BEA 0544B *Coccomyxa sp.* cases, which presented low viability rates but, nevertheless, they experienced a good post-thawing colour evolution (Table 5, fig 4.a). It would be very interesting to set this new validation technique to the requirements of each type of cells and apply the exact staining protocols in future studies.

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• Descripción detallada de las actividades desarrolladas durante la realización del TFT y formación recibida:

Para la realización del TFT las actividades que he desarrollado han sido las siguientes:

- He realizado extracciones de pigmentos para la determinación de cantidad de clorofila mediante técnicas espectrofotométricas.
- He medido la densidad óptica de distintos cultivos en distintas fases de crecimiento, mediante técnicas espectrofotométricas.
- He localizado distintas poblaciones de microalgas mediante el uso de un citómetro de flujo laminar y realizado contaje de células por mililitro para determinar la densidad celular de distintos cultivos.
- He realizado contajes celulares en cámara Thoma para determinar la densidad celular de los cultivos.
- He realizado curvas de crecimiento de las distintas cepas de microalgas con las que iba a trabajar, midiendo cada día distintos parámetros de crecimiento como son: cantidad de clorofila, densidad óptica, número celular por mililitro y peso seco.
- He preparado distintos medios de cultivos sintéticos para mantener las cepas con las que iba a trabajar y he escalado de los cultivos para aumentar la biomasa.
- He preparado soluciones crioprotectoras de concentraciones determinadas (soluciones con Metanol, DMSO y Glicerol)
- He realizado búsquedas bibliográficas y modificaciones de distintos protocolos de criopreservación.
- He realizado búsquedas bibliográficas y modificaciones de distintos protocolos de tinción de células con FDA y Trypan Blue.
- He realizado inmovilizaciones de células en perlas de alginato (encapsulación) tanto en cepas de agua dulce como en cepas de agua salada.
- He sometido a las perlas de alginato a técnicas de deshidratación (vitrificación).
- He realizado búsquedas bibliográficas y modificaciones de distintos protocolos de desencapsulación y recuperación de las células en las perlas de alginato.

• Nivel de integración e implicación dentro del departamento y relaciones con el personal.

En esta práctica he desempeñado mi trabajo como una trabajadora más del centro, he realizado las mismas tareas que cualquier otro científico dentro del Banco Español de Algas sin tener ningún trato especial por ser una alumna de prácticas. El personal del centro en general me ha acogido como a una compañera más, nunca dejando de formarme y ayudarme con todo aquello que necesitara. Por lo general me he sentido muy integrada y bastante cómoda desempeñando mi trabajo.

• Aspectos positivos y negativos más significativos relacionados con el desarrollo del TFT

En general todos los aspectos del desarrollo de este TFT han sido positivos dado que he aprendido a plantear y organizar un experimento científico y a llevarlo a cabo de manera autónoma, siempre contando con la ayuda y supervisión de mi tutora. También he aprendido a los inconvenientes que fueron apareciendo a la hora de realizarlo, de forma independiente. Cabe destacar el buen trato que he recibido por parte de mis compañeras del BEA, quienes me han ayudado siempre que lo he necesitado. Quizás uno de los aspectos negativos es el corto periodo de tiempo con el que conté para llevar a cabo el experimento, que se atrasó aún más tras tener ciertos inconvenientes a la hora de conseguir uno de los materiales necesarios para desempeñar el estudio. Hubiera sido interesante contar con más tiempo para hacer un estudio más profundo y exhaustivo y así poder ampliar y enriquecer el experimento, sin embargo, ajustándome al tiempo del que disponía para realizarlo, considero alcanzados todos los objetivos que se plantearon en un principio.

• Valoración personal del aprendizaje conseguido a lo largo del TFT.

Personalmente estoy satisfecha con el trabajo realizado a lo largo del desarrollo del TFT. Considero que se han alcanzados todos los objetivos fijados en este experimento, con el que he aprendido a trabajar en un laboratorio biotecnológico enfocado a la

biotecnología de microalgas. Gracias a esta asignatura he aprendido a desenvolverme en un ambiente laboral completamente nuevo, he aprendido a planificar y temporalizar los trabajos que se me han asignado así como a subsanar todos los problemas que se han ido planteando a lo largo de la realización del TFT, he afianzado los conocimientos aprendidos durante la carrera respecto a este ámbito y he aprendido nuevos conocimientos más específicos tras la realización de distintas tareas.