Toxicity of Cryoprotectants to Gametophytic Thalli of Red Algae *Porphyra yezoensis*

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Abstract

We assessed the toxicity of cryoprotectant agents (CPAs) to gametophytic thalli of red alga *Porphyra yezoensis* at room temperature. The CPAs used were: dimethyl sulfoxide (DMSO), ethylene glycol (EG), glycerol (GC), 1,2-butanediol (1,2-BD), 1,3-butanediol (1,3-BD), 2,3-butanediol (2,3-BD), 1,3-propanediol (1,3-PD) and propylene glycol (PG). CPA concentrations of 10, 15, 20, 25, 30, 35, 40, 45, and 50% were employed with 30 or 60 s immersion. The toxicity of the eight CPAs to gametophytic thalli of *P. yezoensis* was in the order: 1,3-BD < DMSO $\approx$ 2,3-BD $\approx$ PG $\approx$ EG < GC < 1,3-PD $\approx$ 1,2-BD. All thalli were more sensitive to high CPA concentrations, and most (>75%) thalli survived exposure to 10-25% CPA for 60 s. These data will facilitate selection of the optimal cryoprotectant concentration for cryopreservation of *P. yezoensis* thalli.

Key words: Red algae, *Porphyra yezoensis*, Cryoprotectant agents, Toxicity

Introduction

Cryoprotectant agents (CPAs) are chosen because of their useful non-crystallization property of hydrate formation at low temperatures in aqueous solution (Wowk, 2010). However, the toxicity of which is a fundamental limiting factor for the successful cryopreservation of living cells (Fahy, 2010). So far, most reports of CPA toxicities were focused on fin-fishes such as flounder embryos/sperm (Zhang et al., 2005), zebrafish (Zhang and Rawson, 1993; Liu et al., 2001), medaka (Yang et al., 2010), and so on. However, little research on the toxicity of CPAs to macroalgae has been conducted.

In order to determine the tolerance of gametophytic thalli of *Porphyra yezoensis* to their toxicity without freezing, we used eight CPAs (sulfoxide, diol and triol groups) - dimethyl sulfoxide (DMSO), ethylene glycol (ethane-1,2-diol, EG), glycerol (propan-1,2,3-triol, GC), 1,2-butanediol (1,2-BD), 1,3-butanediol (1,3-BD), 2,3-butanediol (2,3-BD), propylene glycol (1,2-propanediol, PG) and 1,3-propanediol (1,3-PD).

Materials and Methods

Algal strains and culture conditions

Gametophytic thalli of *P. yezoensis* were obtained from TU-1 (Kuwano et al., 1996). Thalli were cultured in a 1 L flask containing Provasoli's enriched seawater medium (Provasoli, 1968) and pieces of synthetic fiber (3-5 cm in length, 0.25 mm in diameter) at 15°C and irradiated at 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with a 10:14 h light:dark photoperiod for two weeks. Monospores were released from thalli attached to the synthetic fibers and grew into young thalli of identical genotype.

CPA toxicity test

Nine concentrations (10, 15, 20, 25, 30, 35, 40, 45 and 50%) of each of the eight CPAs (DMSO, EG, GC, 1,2-BD, 1,3-BD,

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2,3-BD, PG and 1,3-PD) were tested. All chemicals were reagent-grade, and cryoprotectant solutions were prepared in 0.2 µm-filtered seawater. Synthetic fibers with attached thalli were cut to 0.5-1 cm lengths and transferred to CPA solutions for 30 or 60 s. Thalli were then stained with 0.1% erythrosine (in seawater) for 20 min and washed with fresh seawater.

Thalli viability was examined under a light microscope (BX-50; Olympus, Tokyo, Japan). The survival rates were ex-

pressed as the ratio of the number of living thalli to the total number observed.

Results and Discussion

Fig. 1 shows survival of the gametophytic thalli of *P. yezoensis* according to CPA toxicity. The ability of thalli to

Fig. 1. Toxicity of different concentrations of cryoprotectants to gametophytic thalli of *Porphyra yezoensis* illustrated as photograph. DMSO, dimethyl sulfoxide. Scale bars = 50 µm.

survive exposure to CPAs differed, and their tolerance of CPA toxicity tended to decrease with increasing CPA concentration. The thalli showed the highest tolerance to 1,3-BD, and the lowest to 1,2-BD. Glycerol and 1,3-PD were lethal to thalli at 40%, and no thalli survived exposure to 35% 1,2-BD. However, thalli maintained viability (>70%) when exposed to 50% 1,3-BD for 30 s. Therefore, 1,3-BD exhibited the lowest toxicity, followed by DMSO, 2,3-BD, PG and 1,2-BD.

In aqueous solutions, 2,3-BD exhibits glass formation superior to that of other CPA, including 1,2-PD and 1,3-PD (Baudot et al., 1996). Shaw et al. (1995) reported that propanediol forms a stable vitreous state on cooling to a greater degree than other CPA in human and mouse embryos. We found that gametophytic thalli of *P. yezoensis* were highly tolerant of 1,3-BD, even though the determinations were conducted at room temperature.

Fig. 1. Continued.

Selection of a suitable CPA is important for successful cryopreservation. In general, DMSO, GC and methanol have been widely used in algae (Taylor and Fletcher, 1999). DMSO is the most-commonly used CPA for macroalgae, *Porphyra* spp. (Kuwano et al., 1996; Liu et al., 2004; Zhou et al., 2007), *Gracilaria* spp. (van der Meer and Simpson, 1984), and microalgae (Cañavate and Lubian, 1994). Glycerol is a polyol with three hydroxyl groups and is a highly hydroscopic, viscous, odorless, and sweet-tasting fluid of low toxicity (Fluhr

et al., 2008). In this study, glycerol was less toxic than DMSO. Gwo et al. (2005) reported low glycerol toxicity in the microalgae, *Nannochloropsis oculata* at room temperature. CPA toxicity depends on species, developmental stage and storage temperature. In particular, it is known that DMSO is less toxic at 0-5°C than at higher temperatures (Hubálek, 2003). Therefore, the viability of thalli may be enhanced by low temperature preservation. CPA toxicity occurs after permeating into the cell, so the

Fig. 1. Continued.

more toxic CPAs are likely to be more membrane permeable, and *vice versa*. However, greater protection also requires more permeability (Zhang et al., 2005). To date, CPA concentrations before freezing have been considered because they are cryoprotective to cells, but induce cryoinjury in themselves (Fahy, 1986). Moreover, determining the optimum CPA requires a process of trial and error. Therefore, more investigation is required to determine the optimum CPA and its relationship with chemical reactions, temperature, and so on.

References

- Baudot A, Peyridieu JF, Boutron P, Mazuer J and Odin J. 1996. Effect of saccharides on the glass-forming tendency and stability of solutions of 2,3-butanediol, 1,2-propanediol, or 1,3-butanediol in water, phosphate-buffered saline, Euro-Collins solution, or Saint Thomas cardioplegic solution. *Cryobiology* 33, 363-375.
- Cañavate JP and Lubian LM. 1994. Tolerance of six marine microalgae to the cryoprotectants dimethyl sulfoxide and methanol. *J Phycol* 30, 559-565.
- Fahy GM. 1986. The relevance of cryoprotectant "Toxicity" to cryobiology. *Cryobiology* 23, 1-13.
- Fahy GM. 2010. Cryoprotectant toxicity neutralization. *Cryobiology* 60 (3 Suppl), S45-S53.
- Fluhr JW, Darlenski R and Surber C. 2008. Glycerol and the skin: holistic approach to its origin and functions. *Br J Dermatol* 159, 23-34.
- Gwo JC, Chiu JY, Chou CC and Cheng HY. 2005. Cryopreservation of a marine microalgae, *Nannochloropsis oculata* (Eustigmatophyceae). *Cryobiology* 50, 338-343.
- Hubálek Z. 2003. Protectants used in cryopreservation of microorganisms. *Cryobiology* 46, 205-229.
- Kuwano K, Aruga Y and Saga N. 1996. Cryopreservation of clonal gametophytic thalli of *Porphyra* (Rhodophyta). *Plant Sci* 116, 117-124.
- Liu H, Yu W, Dai J, Gong Q, Yang K and Lu X. 2004. Cryopreservation of protoplasts of the alga *Porphyra yezoensis* by vitrification. *Plant Sci* 166, 97-102.
- Liu XH, Zhang T and Rawson DM. 2001. Differential scanning calorimetry studies of intraembryonic freezing and cryoprotectant penetration in zebrafish (*Danio rerio*) embryo. *J Exp Zool* 290, 299-310.
- Provasoli L. 1968. Media and prospects for the cultivation of marine algae. In: *Cultures and Collections of Algae, Proceedings of U.S. Japan Conference*. Watanabe A and Hattori A, eds. Japanese Society of Plant Physiology, Kyoto, JP, pp. 63-75.
- Shaw JM, Ward C and Trounson AO. 1995. Survival of mouse blastocysts slow cooled in propanediol or ethylene glycol is influenced by the thawing procedure, sucrose and antifreeze proteins. *Theriogenology* 43, 1289-1300.
- Taylor R and Fletcher RL. 1999. Cryopreservation of eukaryotic algae: a review of methodologies. *J Appl Phycol* 10, 481-501.
- van der Meer JP and Simpson FJ. 1984. Cryopreservation of *Gracilaria tikvahiae* (Rhodophyta) and other macrophytic marine algae. *Phycologia* 23, 195-202.
- Wowk B. 2010. Thermodynamic aspects of vitrification. *Cryobiology* 60, 11-22.
- Yang H, Norris M, Winn R and Tiersch TR. 2010. Evaluation of cryoprotectant and cooling rate for sperm cryopreservation in the euryhaline fish medaka *Oryzias latipes*. *Cryobiology* 61, 211-219.
- Zhang T and Rawson DM. 1993. Cryoprotectant toxicity and permeability studies on zebrafish (*Brachydanio rerio*) embryos. *Cryobiology* 30, 640.
- Zhang YZ, Zhang SC, Liu XZ, Xu YJ, Hu JH, Xu YY, Li J and Chen SL. 2005. Toxicity and protective efficiency of cryoprotectants to flounder (*Paralichthys olivaceus*) embryos. *Theriogenology* 63, 763-773.
- Zhou W, Li Y and Dai J. 2007. Study on cryopreservation of *Porphyra yezoensis* conchocelis. *J Ocean Univ China* 6, 299-302.