Does the accumulation of lipid droplets and carotenoids affect the viability of *Dunaliella salina* cells after cryopreservation?

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Microalgae have garnered significant attention in recent years due to their diverse applications in various fields, including biotechnology, biofuels, nutraceuticals, and environmental monitoring. Among these microalgae, *Dunaliella salina* stands out as a species of immense biotechnological importance. Its unique characteristics and potential applications have prompted researchers to explore methods of preserving this microalga effectively, with cryopreservation being a particularly relevant approach.

In our previous studies, we demonstrated that *Dunaliella salina* cells are capable of accumulating significant amounts of carotenoids and lipids in response to salt and temperature stress, altering the pigment composition based on cooling regimes (Chernobai 2022, 2023). In this study, we attempted to address the question of whether the accumulation of lipid droplets and carotenoids affects the viability of cells after cryopreservation.

It has been shown that cooling to -40°C followed by immersion in liquid nitrogen without the use of cryoprotectants significantly reduces the concentration and mobility of cells after thawing compared to intact culture.

To enhance cell viability after cooling-thawing, cold adaptation ("hardening") was applied for 24 hours at a temperature of 4°C, and 10% DMSO, glycerol, or ethylene glycol were used as cryoprotectants.

Cryopreservation of pre-"hardened" *Dunaliella salina* cells with the addition of cryoprotectant solutions significantly improved viability (up to 80% compared to control) indicators after cooling-thawing. The best viability results at all stages of the experiment were achieved using cells cultivated under stress conditions (with nutrient deficiency and elevated sodium chloride content) and with the use of 10% DMSO and glycerol as cryoprotectants.