Cryopreservation of cyanobacteria in the BCCM/ULC collection: experimental set-up of protocols

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Cryopreservation is considered as the preferred method for the long-term storage of many micro-organisms, including cyanobacteria. The BCCM/ULC collection is now holding over 240 cyanobacterial strains but only 62 are maintained in a cryopreserved state. The development of improved cryopreservation protocols is therefore required both for the future growth and valorization of the collection.

We have evaluated two methods as potential long-term preservation techniques: (i) the two step cooling method, the common freezing procedure of algae, and (ii) the encapsulation-dehydration method, often considered as a promising alternative to the traditional cryopreservation method for recalcitrant microalgal strains.

The effects of several factors on the viability of four representative cyanobacterial strains for both methods were first considered to determine which ones are the most important for a successful cryopreservation. For the two step method, these factors include the cryoprotectant choice, the sample preparation methods (e.g. direct growth inside the cryovials) and the growing periods of the cultures tested. For the encapsulation-dehydration method, several cryoprotectants, evaporative drying methods and dissolution solutions of the alginate beads were considered. We compared the storage at -70°C and in liquid nitrogen with both methods. A vital staining method allowing the rapid evaluation of the post-cryopreservation viability was also assessed.

Based on the results for the four strains, an optimized cryopreservation protocol was developed for both methods and tested on another set of 26 cyanobacterial strains. Most of the strains displayed high survival rates using the two-step cryopreservation protocol but only a few survived the encapsulation-dehydration process. Our results demonstrated that cryopreservation by the traditional two-step cooling procedures was effective for cyanobacterial strains having various morphologies and origins.