Development of cryopreservation methods for long-term preservation of cyanobacterial strains in the BCCM/ULC collection

Charlotte Crahay\(^1\), Marine Renard\(^1\), Maud Mari\(^1\), John G. Day\(^2\), Annick Wilmotte\(^1\)

\(^1\) University of Liège, Centre for Protein Engineering, Allée de la Chimie 13 B6a, 4000 Liège, Belgium, \(^2\) Culture Collection for Algae and Protozoa, Scottish Marine Institute, Oban, Argyll PA37 1QA, UK

Long-term genetic and functional stability is a fundamental requirement for the maintenance of microorganisms and cryopreservation is the preferred method for the long-term storage of many micro-organisms, including cyanobacteria. The BCCM/ULC collection currently holds 200 cyanobacterial strains, but only 62 are cryo-preserved. The main limiting factors are the low levels of survival of some strains and the long periods required to recover from cryopreservation, and thus the inability to deliver rapidly cryopreserved strains to the user community. The development of improved cryopreservation protocols is therefore required for the future expansion and valorization of the collection. The BRAIN-be project PRESPHOTO (preservation of photosynthetic micro-algae in the BCCM collections) (www.presphoto.ulg.ac.be) aims to improve the preservation of cyanobacterial and diatoms in the BCCM/ULC and BCCM/DCG collections, respectively.

Both traditional two-step cooling and the encapsulation-dehydration methods were evaluated as potential long-term preservation techniques. The effects of several factors on the viability of 4 strains have been investigated to determine which are the most important for their successful cryopreservation. These include cryoprotectant choice and sample preparation methods (e.g. direct growth inside the cryovials) of the cultures tested. We have also compared storage at -70°C and in liquid nitrogen (-196°C). In addition, a vital staining method, allowing the rapid evaluation of post-cryopreservation viability has been assessed.

In the final phase of the project, the selected cryopreservation protocols will be tested on a large set of strains. In addition, an independent validation of the protocols will be performed by both partners of the project (BCCM/DCG and CCAP).