

INTER-LABORATORY EVALUATION, DEVELOPMENT AND VALIDATION OF FUNGAL PRESERVATION REGIMES USED IN DIFFERENT EUROPEAN BIOLOGICAL RESOURCES CENTRES (BRCS)

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Successful preservation of fungi relies on the application of optimised preservation protocols that do not compromise the genomic integrity of the organism. Most major European BRCs use lyophilisation and cryopreservation as the methods of choice. Although based on generic principals, protocols can vary between institutions and do not always result in successful recovery. In order to evaluate the efficacy of the methods, a range of fungal strains were circulated around partner collections in the EMbaRC project and the organisms preserved using the standard methods used in each collection. The effectiveness of preservation was assessed using a series of techniques including DNA fingerprinting and sequencing, analysis of culture characteristics, viability assessments and the use of MALDI-TOF. The results showed that when viable cultures were obtained after preservation, they appeared to retain their genomic integrity, but there was evidence of delayed growth and attenuation in some cultures. Not all fungi were successfully preserved by all methods. It was found that a cryopreservation protocol used by the MUCL collection in Belgium, that limited manipulation of the fungus before preservation, was particularly effective in preserving some of the more delicate fungi and this method is being evaluated by the project partners.

A further investigation was undertaken to assess the integrity of four specific strains of fungi deposited in different collections. They were compared using culture analysis, sequence analysis, DNA fingerprinting and MALDI-TOF. It was found that some limited variation was observed at the phenotypic level from the analysis of culture characteristics, but this could be expected, especially in organisms such as *Aspergillus* which can be prone to strain drift. More importantly, molecular integrity remained consistent with no significant differences observed between lines of the same strain. Therefore, despite the strains having been maintained by different methods over the intervening years from their initial deposit, the collections had maintained them in a stable manner. This is indicative of the benefits of applying proven regimes, resulting in high quality operations.

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