MICRO BIOTEC 15

Congress *of* Microbiology *and* Biotechnology

December **2015**

University of Évora

EDITOR
Ana Teresa Caldeira, António Candeias, António Pereira and M. Rosário Martins
HERCULES Laboratory

GRAPHIC DESIGN
Nuno Carriço & Vanda Amaral

PRINT
GRECA – Artes Gráficas, Lda

ISBN
978-989-99475-2-8

Retrieval and molecular identification of 60 year old *Candida* clinical isolates

C. Soares[1], M.L. Martins[2], A.P. Sampaio[3], C. Pais[3] and N. Lima[1]

(1) Centre of Biological Engineering, Micoteca da Universidade do Minho, Portugal.
(2) Laboratório de Micologia, UEI de Microbiologia Médica, IHMT, UNL,Portugal.
(3) Centre of Molecular and Environmental Biology (CBMA), University of Minho, Portugal.

The Institute of Hygiene and Tropical Medicine of Lisbon provided to the fungal culture collection Micoteca da Universidade do Minho (MUM) a set of freeze-dried glass ampoules with different fungal clinical samples preserved between 1953 and 1955 (Fig. 1). The goal of this contribution was to allow MUM to check the viability of the strains, retrieval the ones that remained viable, and confirm their identity using modern molecular approaches. The final objective is to give once again access to the end-users, through the MUM e-catalogue, these authenticated biological materials with reproducible properties that will allow them to use these strains to fit their needs.

The received ampoules had no information about the freeze-drying conditions or preservatives used and were already 60 year old, while viability described for viable freezedrying storage is described as 20-40 years. Since then, freeze-drying has had multiple studies and the technology has evolved. Alongside, the fungal taxonomy and identification approaches have changed enormously. With this in mind 44 ampoules with strains of 21 species of *Candida* were opened and their viability and identification were tested.

The revival procedure consisted in rehydrating the ampoules using malt extract-glucose yeast extract-peptone medium for 24 hours. An aliquot was then smeared in the same agarised medium and incubated at 30 ºC. The remaining material inside the ampoule was also incubated at 30 ºC. All plates and ampoules were observed daily and all visible growth analysed and transferred to new plates.

Results showed that 29 (66%) of the freeze-dried strains were not viable. However, 15 strains identified as belonging to 11 different species (*C. albicans, C. flareri, C. guillermondi, C. krusei, C. ludwigi, C. macedoniensis, C. manitholphermentans, C. parabalcanica, C. parapsilosis, C. stellatoideia, C. tumefaciens*) remained viable showing good resilience to the long-term freeze-drying preservation.

The retrieved strains were then analysed by sequencing of the entire ribosomal ITS region (i.e., ITS1/5.8S rDNA/ITS2) and the results obtained were compared with the previous identifications. At the moment, 6 of the retrieved strains had its identification confirmed by ITS sequencing, while 5 have been renamed. The 4 remaining strains still need further analysis to confirm their identification.

Figure 1: Example of an old freeze-drying ampoule and the associated information.