

MICRO
BIOTECH|15

**Congress of
Microbiology and
Biotechnology**

December 2015
University of Évora

EDITOR

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GRAPHIC DESIGN

Nuno Carriço & Vanda Amaral

PRINT

GRECA - Artes Gráficas, Lda

ISBN

978-989-99475-2-8

Polyphasic identification of a *Rhizopus* species and a putative novel *Gongronella* which both degrade the fungicide metalaxyl

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Mucorales are mostly ubiquitous terrestrial filamentous fungi with coenocytic hyphae and asexual reproduction based on specialized structures (sporangia) that contain few to several thousand sporangiospores. Some of them have an important role as decomposers. The ability of some of them to degrade xenobiotic and recalcitrant compounds is an economically significant property, making them useful for bioremediation processes.

A previously unidentified *Gongronella* strain and a *Rhizopus stolonifer* were isolated from vineyard soil from Alentejo, Portugal. These two strains showed high tolerance of and ability to, degrade the acyl-alanine fungicide, metalaxyl [1] after being selectively enriched with the compound.

Several Mucorales reference species from *Absidia*, *Circinella*, *Gongronella* and *Rhizopus* were included in this present polyphasic study to identify the *Gongronella* to species and confirm the identity of the *R. stolonifer*. The approach combined macro- and micromorphology, sequence analysis of the entire ribosomal ITS region (i.e., ITS1/5.8S rDNA/ITS2) and Matrix Assisted Laser Desorption Ionization Time-of-Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS [2]). The *R. stolonifer* was reidentified as *Rhizopus oryzae* due to a high ITS and MALDI-TOF ICMS similarity with the reference strains.

The macromorphological results determined that the *Gongronella* was distinct from *Gongronella lacrispora*. When compared with *Gongronella butleri*, it showed similar cultural traits but in the micromorphology the sporangia are smaller. The spore shape was fusiform in contrast with the ovoid and tear-shaped spores of *G. butleri* and *G. lacrispora*, respectively. The ITS sequence grouped the *Gongronella* strain with *G. butleri* and *G. lacrispora*, forming a distinct cluster from the other Mucorales studied. However, *G. butleri*, *G. lacrispora* and the *Gongronella* strain were well separated within this cluster which was corroborated by MALDI-TOF ICMS. The results indicate that the *Gongronella* strain is a putative novel species that will be described formally in future work.

[1] M. R. Martins, P. Pereira, N. Lima, J. Cruz-Morais, Archives of Environmental Contamination and Toxicology, 65, 2013, 67.

[2] C. Santos, R. M. Paterson, A. Venâncio, N. Lima, Journal of Applied Microbiology, 108, 2010, 375