Polyphasic approach for the identification of Zygomycota strains with Biotechnological use M. Martins⁴⁻⁵, V. Dantas⁴⁻⁵, C. Soares³, C. Santos², G. Verkley⁶, J. Cruz-Morais⁴⁻⁵, N. Lima³⁻¹ Post-Graduate Programme in Agricultural Microbiology, Federal University of Lavras, Lavras, Brazil Facultad de Ingeniería y Ciencias, Universidad de La Frontera, 4811-230 Temuco, Chile Centre of Biological Engineering, Micoteca da Universidade do Minho, Braga Departamento de Química, Escola de Ciências e Tecnologia ICAAM- Instituto de Ciências Agrárias e Mediterrânicas, Universidade de Évora, Évora, Portugal CBS-KNAW, Fungal Biodiversity Centre, Utrecht. The Netherlands

Mucorales (Zygomycota) are mostly terrestrial ubiquitous filamentous fungi with coenocytic hyphae and asexual reproduction based on specialized structures (sporangia) that contain few to several thousand of sporangiospores. In addition, as result of sexual reproduction the zygosporangia, where resistant zygospores are formed, gave the name to this group. The natural relationships of Zygomycota are very poorly understood due to the lack of distinguishing morphological characters. Nevertheless their important role in nature as decomposers, especially the ability to degrade xenobiotic and recalcitrant compounds, make them important candidates for biotechnological use in the bioremediation processes.

In previous work, two strains, Gongronella sp. CCMI 1101 (MUM 10.263) and Rhizopus stolonifer, CCMI 1105 (MUM 10.260), isolated from a Portuguese (Alentejo) vineyard soil, were used to perform a selective adaptation to metalaxyl, an acylalanine fungicide widely used against oomycetes causing downy mildews. Gongronella sp. CCMI 1100 (MUM 10.262) and R.stolonifer CCMI 1104 (MUM 10.261) showed high tolerance and capacity to degrade metalaxyl [1].

In this study, in order to better characterize the non-adapted and metalaxyl adapted strains, we assessed these strains and a selection of other strains from the genera *Gongronella*, *Absidia*, *Circinella* and *Rhizopus*, using a polyphasic approach combining classical morphology, molecular biology and Matrix Assisted Laser Desorption Ionization Time of Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS), as described elsewhere [2]. The results show that in the ITS phylogeny analysis *Rhizopus stolonifer* is *Rhizopus oryzae* and *Gongronella* sp. were grouped with the closely related species *G. butleri* and *G. lacrispora*. In addition, the spectral analysis confirm these strains as *Rhizopus oryzae*, grouping with the other strains from that species. MALDI-TOF ICMS and M13-PCR fingerprinting were not able to separate the non-adapted to metalaxyl adapted strains.

At the moment, from the molecular and proteomic analyses CCMI 1100 seems to represent a putative new species of *Gongronella*.



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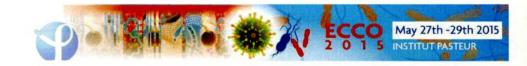
European Culture Collections as tools in research and biotechnology

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