Characterisation by molecular biology and MALDI-TOF ICMS of zygomycete Gongronella sp. isolated from an Alentejo vineyard soil

M. Rosário Martins¹, Cledir Santos², Pablo Pereira³, Fernanda Simões⁴, Júlio Cruz-Morais¹, Nelson Lima²

¹Chemistry Department & ICAAM, School of Science and Technology, University of Évora, Évora, Portugal; ²IBB/ Centre of Biological Engineering, University of Minho, Braga, Portugal; ³UITA - Unidade de Investigação de Tecnologia Alimentar, L-INIA, INRB, Lisboa, Portugal; ⁴Molecular Biology Group, UIRGEMP, L-INIA, Instituto Nacional de Recursos Biológicos, Lisboa, Portugal.

Zygomycete Gongronella sp. CCMI 1101 (MUM 10.263) was isolated from an Alentejo vineyard soil submitted to repeated treatments with the fungicide metalaxyl. This fungicide is widely used against Oomycetes species that causing downy mildew. After selective enrichment, adapted Gongronella sp. CCMI 1100 (MUM 10.262) showed high tolerance to metalaxyl with EC₅₀ of 36.1 mg L⁻¹. Additionally, this adapted fungus was able to use metalaxyl as main carbon and energy source in liquid cultures containing 10 and 50 mg L⁻¹ with a degradation rate of 0.119±0.001 and 0.386±0.002 mg L⁻¹ day⁻¹, respectively. Afterwards, both Gongronella strains (non-adapted and metalaxyl adapted strains) were submitted to a polyphasic approach identification and characterisation. To perform this, morphology characterisation, ITS sequence region and Matrix Assisted Laser Desorption Ionization Time of Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS) were carried out. In order to differentiate soil strains Gongronella sp. from adapted strains M13-PCR typing and proteomic using MALDI-TOF ICMS were used. The ITS phylogeny tree and MALDI-TOF ICMS dendrogram both corroborate that Gongronella sp. grouped with related species G. butleri and G. lacrispora. However, it seems to be a putative new species. Additionally, no differences of non-adapted and adapted Gongronella sp. strains were observed by M13-PCR typing. In contrast, proteomic analysis showed spectra with substantial quantitatively phenotypic differences between both strains that could be a consequence of a strain adaptation to metalaxyl. In conclusion, Gongronella sp. CCMI 1101 is now under further taxonomic studies and the current results suggest that the adapted strain CCMI 1100 can be explored in soil bioremediation for metalaxyl-degradation.