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Characterisation of Filamentous Fungal Diversity of *Trichoderma* Genus Isolated from Marine Sponge by Sequencing ITS-rDNA and MALDI-TOF ICMS

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Filamentous fungi are ubiquitous organisms and can easily be isolated from a wide range of environment. Some efforts have been made to investigate microbial communities in marine sponges of eukaryotes using both approaches of conventional taxonomy (morphology) and molecular taxonomy (Passarini *et al.* 2011). The classification of some filamentous fungi taxa can be difficult to achieve based on these methods. Currently, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS) has been widely used to identify and characterise a large number of taxa of filamentous fungi (Santos *et al.* 2010). The aim of this study was to investigate the diversity of 33 *Trichoderma* isolated from the southern Atlantic Ocean marine sponge *Dragmacidon reticulata* using a polyphasic approach. The polyphasic approach was based on molecular biology (ITS-rDNA gene region), macro- and micro-morphology and spectral analyses using MALDI-TOF ICMS. Through ITS-rDNA gene region sequencing five species, namely *T. viride* (1 isolate, 3%); *T. atroviride* (8 isolates, 24%); *T.* cf. *stilbohypoxyli* (1 isolate, 3%); *T. asperellum* (4 isolates, 12%); and *T. harzianum* (19 isolates, 58%) were putatively identified (Fig. 1).

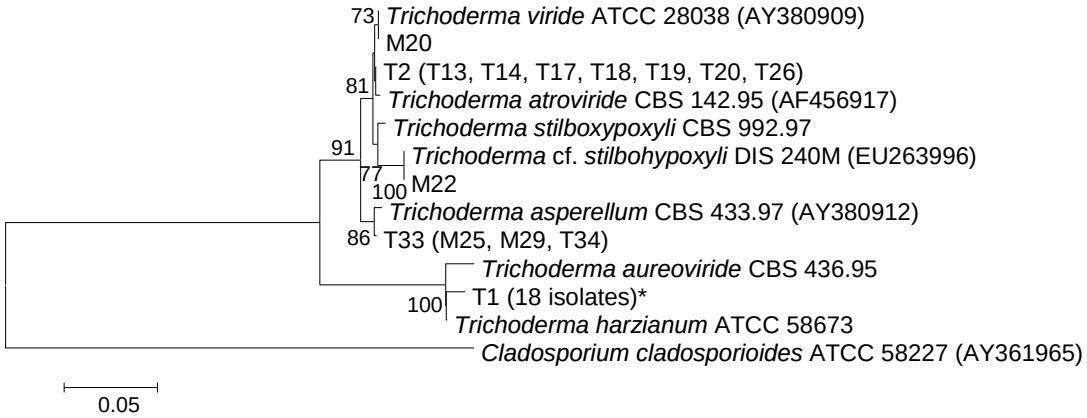

Figure 1. *Trichoderma* phylogenetic tree based on ITS analyses showing closest relatives of filamentous fungi isolated from marine esponge (Kimura two-parameter model; Neighbor-Joining algorithm and 1,000 replicate bootstrap). (*T3, T5, T6, T9, T11, T12, T15, T16, T21, T23, T24, T25, T28, T32, T35, T36, T37, T38).

At macro- and micro-morphologies point of view 19 isolates were identified as *T. harzianum*, 1 isolate (3%) as *T. stromaticum*, and 1 isolate (3%) as *T. viride*. The remains 12 isolates (36%) were not identified through the classical approach either did

not present reproductive structure or presented ambiguous traits (data not shown). Finally, the spectral analyses by MALDI-TOF ICMS after statistic analyses using SARAMIS™ software (Spectral Archiving and Microbial Identification System, AnagnosTec, Germany) grouped the isolates according to the molecular results very well (Fig. 2).

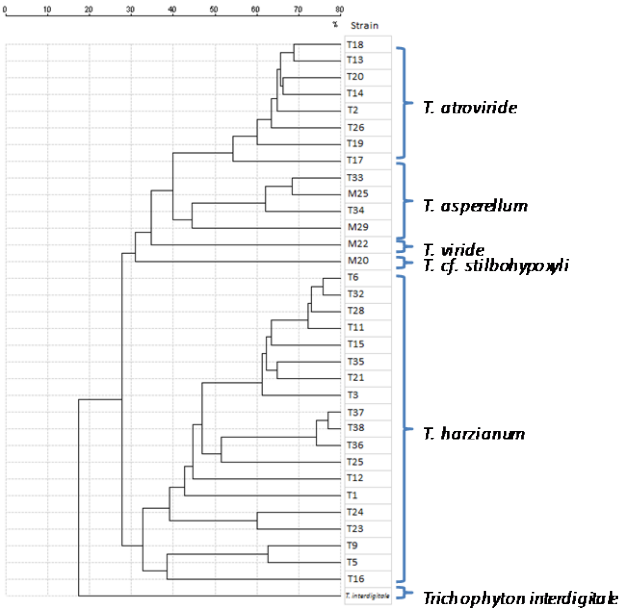

Figure 2. Dendrogram obtained by MALDI-TOF ICMS analyses of 33 strains of *Trichoderma* isolates from marine sponge. *Trichophyton interdigitale* was used as out group.

Although the SARAMIS™ database contains records of *Trichoderma* spectra of the species analysed, in this particularly work, no match was achieved between these spectra and the spectra obtained from our isolates. More analyses need to be done using different media and growth conditions to overcome these discrepancies between data in our database. The MALDI-TOF ICMS was the faster technique between the three tools used in this polyphasic approach. However, the overall results show that spectral approach using MALDI-TOF ICMS needs to be supported by molecular biology analyses because the spectral database does not covers all growth conditions and the whole biodiversity.

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