



Real or fake friends?

Study of *Trichoderma* strains in Brazilian stingless bee hives

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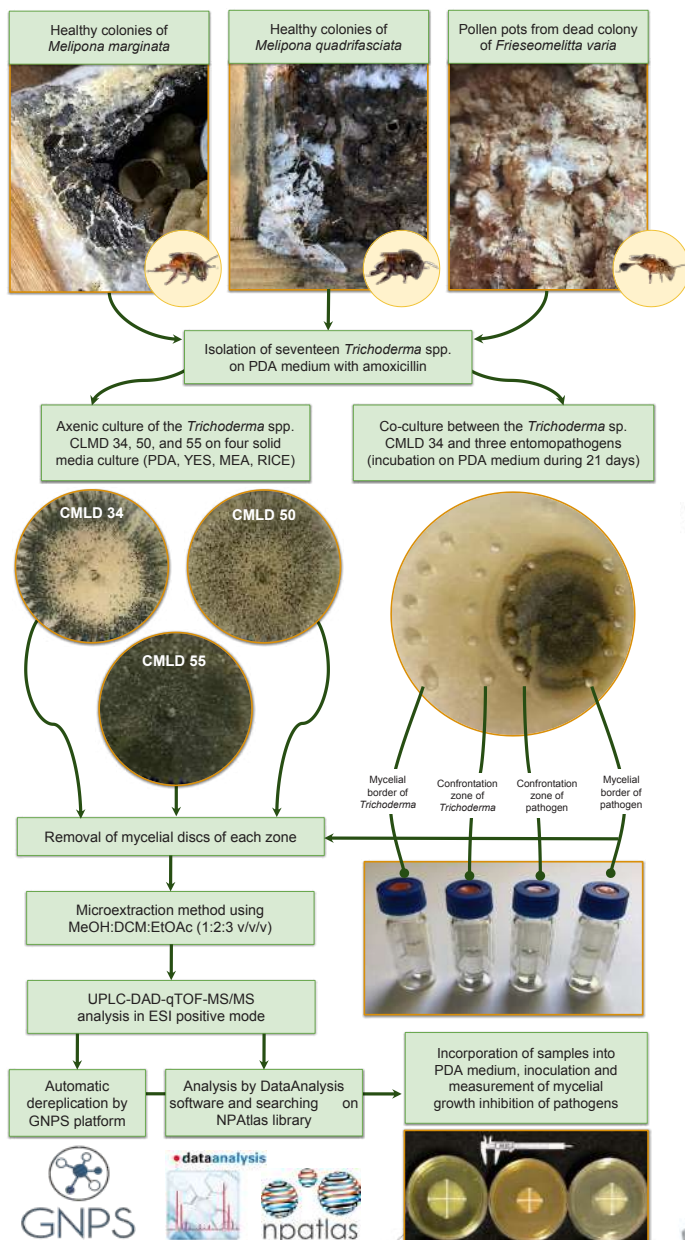
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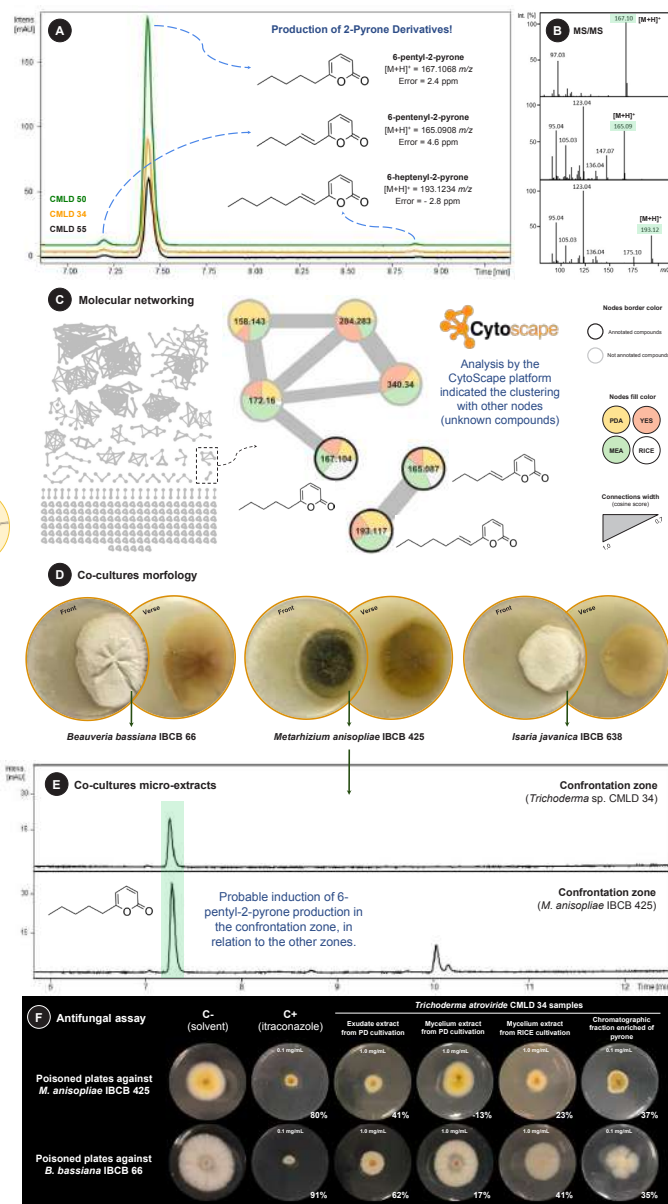
INTRODUCTION AND OBJECTIVE

The genus *Trichoderma* are usually present as components of biological control products for agricultural systems, besides being naturally found colonizing many terrestrial substrates like in bee environments. Although they are not considered bee pathogens, some *Trichoderma* species have recently been reported to significantly reduce the survival of honey bees. During the ongoing study, the predominance of *Trichoderma* spp. was observed in different parts of three stingless bee colonies. This stimulated the chemical ecology investigation about these fungi in the bee environment.

MATERIALS AND METHODS



RESULTS AND DISCUSSION



Figures A-F. Results about the *Trichoderma* spp. strains. A) Chromatograms (filtered in 300 nm) of the three axenic incubation of *Trichoderma* spp. strains growing during 7 days on PDA medium. B) Fragmentation spectrum (MS/MS) from the three 2-pyrone derivatives dereplicated. C) Molecular networking obtained from MS/MS data of micro-extracts of the strain CLMD 34. D) Pictures of co-cultures between the CLMD 34 and the three entomopathogens. E) Comparison of chromatograms (filtered in 300 nm) of the co-culture between CLMD 34 and *M. anisopliae*, enclosing on induction of the 6-pentyl-2-pyrone. F) Antifungal susceptibility test against the three entomopathogens of chemical samples (extracts and fractions) obtained from the cultivation of strain CLMD 34.

CONCLUSION AND NEXT STEPS

The major production of 2-pyrone derivatives by *Trichoderma* spp. associated with stingless bees, added to their knowledge of having antimicrobial activities, made this group of compounds a promising target in ongoing studies. The detected metabolites will be investigated in toxicity tests against stingless bees. Thus, with the obtained results this work intends to assist the elucidation of the ecological roles of filamentous fungi associated with stingless bees through the comprehension of their metabolomic arsenal.

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The fungal companions of stingless bees are still a mystery. The environment of stingless bee colonies, with its homeostatic conditions and abundance of nutritional resources, favors microbial growth. Some interactions between fungi (*Zygosaccharomyces*) and stingless bees (*Melipona scutellaris*) have already demonstrated the beneficial partnership between the organisms involved. However, other antagonistic interactions involving fungal entomopathogens of bees are considered a risk to the productivity of beekeeping and meliponiculture. During the management of Brazilian hives of the species *manduri* (*Melipona marginata*), *mandaçaia* (*Melipona quadrifasciata*) and *marmelada* (*Frieseomelitta varia*), the predominance of mycelial growth was observed. These fungi were found growing on the batume and wooden box of healthy *Melipona* hives, but were not observed colonizing the internal resources of the colonies. Only in the dead colony of marmalade was it possible to observe macroscopically the growth of the same fungus in the pollen pots. The cultivation-dependent techniques allowed the obtaining of twelve strains phylogenetically identified to the fungal genus *Trichoderma*, with a predominance of the species *T. atroviride*. The analysis of microextracts of axenic cultures of *Trichoderma* spp. strains by HPLC-HRMS/MS combined with metabolomic approaches indicated the production of pyrone derivatives by the microorganisms. Subsequent tests with *Trichoderma atroviride* CLMD 34 in cellular (co-cultures) or chemical (antifungal susceptibility assays) antagonism assays against three entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria javanica*) highlighted the possible role of pyrones as a modulating agent of ecological interaction. This secondary metabolite is currently being isolated, characterized and tested to understand whether it has any role in the colony environment. If *Trichoderma* spp. acts as a beneficial symbiont for bees, the accumulation of these metabolites in the hive microenvironments could prevent the proliferation of entomopathogenic agents. However, the limitation of *Trichoderma* growth mainly in the external substrates of the hive may indicate its saprotrophic commensal ecological character. The current work investigates the possibility of *Trichoderma* strains acting as a harmonious symbiont, with the aim of elucidating its still uncertain roles both for stingless bees and for meliponiculture.

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