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Isolation of *Trichoderma spp*. from Malta island and alternative method of preservation

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Introduction

The Maltese Islands are located in the central Mediterranean sea and have a strongly bi-seasonal climate characterised by mild, wet winters and hot, dry summers. The average annual precipitation is 530 mm. *Trichoderma spp*. are endophytic, soil-borne fungi, green-spored filamentous ascomycetes and are found world wide. This genus was first described by Persoon 1794 and Tulasne 1865. According with Druzhinina et al. 2011, to date at least 1100 strains of *Hypocrea* (sexual telomorphic stage), and Trichoderma (asexual anamorphic stage), have been identified, and many new species are being recognized. *Trichoderma spp*. are widely studied due to their uses as mycoparasites biocontrol agents. Furthermore, they stimulate plant growth, suppress plant diseases by one or more different direct and/or indirect mechanisms and solubilize nutrients. Some strains can induce host growth under abiotic stress, and improve seed germination under saline conditions. These fungi are producers of a number of secondary metabolites with antibiosis properties and they also have several biotechnological applications. On the other hand, *T. harzianum*, *T. longibrachiatum* and *T. viride* have been recognized as opportunistic pathogens of immunocompromised persons. Trichoderma is a powerful organism to study for its capacity of helping good practise against climate change. Since, few studies on *Trichoderma spp*. are recorded on the Maltese Island, the present work was aimed to investigate presence, isolation and preservation on different substrates.

Materials and Methods

Five different locations were randomly selected in Malta and 2.5kg of soil was collected for each location with a sterile shovel and stored in a closed clean bucket.

<u>Isolation from soil.</u>
The soil (1gr) was mixed with 1L of sterile distilled water in a sterile glass bottle. Serial dilutions were performed and 20µl of each dilution was spread on 4 petri dishes (Ø = 9cm) with modified Trichoderma Selective Medium (TSM). The petri dishes were incubated at 26±1 C°, with a light to dark cycle 16L:8D. After 48hrs the cultures were inspected for the presence of *Trichoderma spp*.

<u>Isolation from other substrates.</u>
A green moldy fungus was observed growing on different substrates such as (1) on commercial compost, (2) on coffee grounds, (3) on *Poliporacea sp*. on *Prunus cerasifera*, (4) on dead branch of *Euphorbia grandis*.

<u>Preservation.</u>
Monocondial isolation was performed and the isolates were preserved in 99% Glycerol, Sterile distilled water, Sintetic Nutrient Agar SNA, PDA and sterile coffee ground.


1.


2.


3.

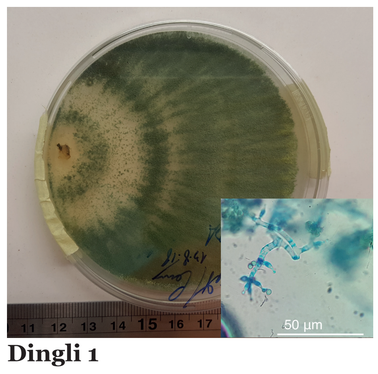

4.

Figures. (1) *Trichoderma* growing on commercial compost; (2) *Trichoderma* growing on *Poliporacea sp*. on *Prunus cerasifera*; (3) *Trichoderma* growing on coffee ground; (4) *Trichoderma* growing on *Euphorbia grandis*.

Results

<u>Isolation from soil.</u>

Trichoderma spp. were isolated in 4 different soil samples collected in Floriana, North of Malta and two locantions in Dingli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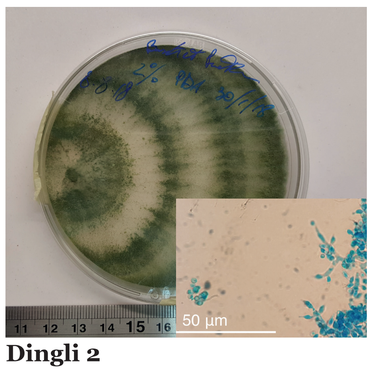
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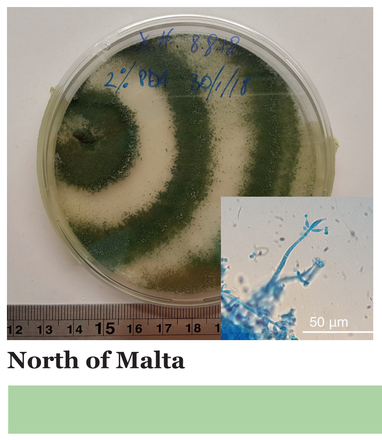
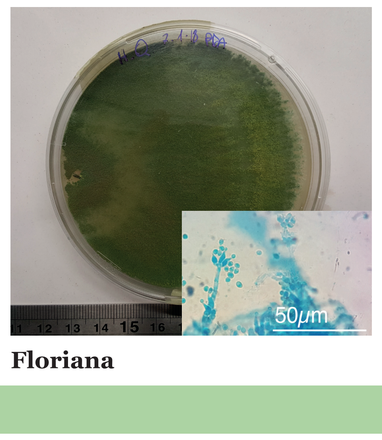
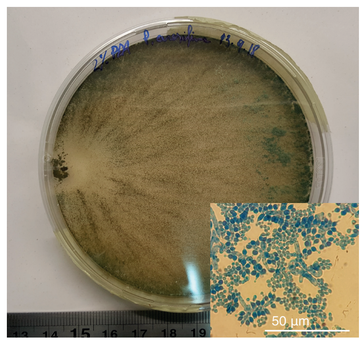
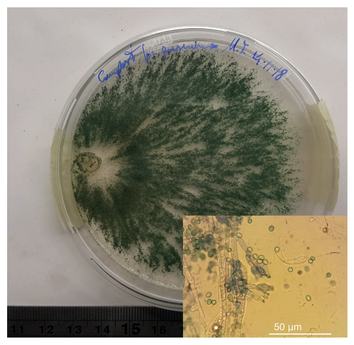

North of Malta

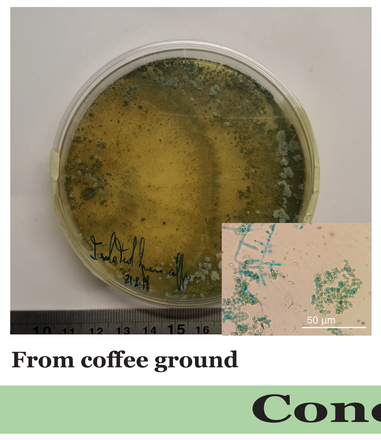

Floriana

<u>Isolation from other substrates.</u>

The green moldy fungus growing on compost, coffeground, deadbranch of *Euphorbia grandis*, and *Poliporacea sp*. on *Prunus cerasifera* were confirmed to be *Trichoderma spp*.


Poliporacea sp. on *Prunus cerasifera*


From commercial compost


From coffee ground

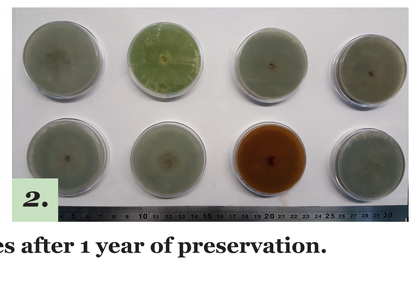

Dead branch of *Euphorbia sp*.

<u>Preservation.</u>

Pure culture of *Trichoderma* isolates. were preserved in 5 different media namely 99% Glycerol, sterile distilled water, SNA, PDA and sterile coffee grounds. Storage temperature was at 4°C except for glycerol and coffe grouds. The viability of the preserved colture was tested after 1 year with results shown in the table below.

Substrates	Glycerol	PDA	SNA	H_2O	Coffee grounds
Temperature	-18°C	4°C	4°C	4°C	Room T°
Spores	✓	✓	✓	✓	✓
Mycelia	×	✓	✓	×	✓


1.


2.

Viability test of *Trichoderma* isolates after 1 year of preservation.
(1) Top side (2) Bottom side.

Conclusion

Trichoderma spp. have been isolated from soils and other substrates, confirming their presence on the Maltese Island. The technique of monocondial isolation is a useful tool to produce axenic cultures of *Trichoderma spp*. although it is strongly suggested to perform more than one monocondial isolation in order to preserve more genetic diversity. Axenic cultures of indigenous *Trichoderma spp*. have been preserved at Argotti Botanic Garden and Resource Centre. Moreover, they may be studied for their biotechnological applications .

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