

Bioindicators in Forensic science.

Marco Iannaccone¹, Silvia Giusto¹, Grazia Cecchi¹, Simone Di Piazza¹, Mirca Zotti¹

¹Laboratory of Mycology, DISTAV, University of Genoa, C.so Europa, n°26, 16132 Genoa, Italy.

marco.iannaccone@edu.unige.it

Introduction

- Bioindicators encompass biotic and abiotic responses to ecological change and, include taxa from multiple Kingdoms used to monitor environmental conditions and temporal changes. They are applied in various biotechnological fields, including Forensic science, where they help identify natural traces and estimate post-mortem intervals (PMI).
- Forensic mycology is an emerging and largely unexplored field. The Natural Trace project, titled “Fungi as forensic tools” and funded by the Marie Skłodowska-Curie Actions of the European Commission, seeks to fill this research gap.
- The project investigates fungal presence/absence, diversity, and growth on forensic-relevant materials. A model crime scene was created using a garment buried in soil for 1 month, then stored under controlled conditions for over a year to simulate a burial site.
- Fungi colonizing the garment were isolated axenically, their growth rates measured and identified through DNA barcoding. These isolates were securely preserved in the culture collection of DISTAV (CoID-UNIGE), which is part of MIRRI-IT and a project partner of SUS-MIRRI.IT.

Material and Methods

- Techniques deployed to sample: swab and direct scratching.
- Samples taken from a buried shirt and the plastic bag in which the shirt was contained.
- Direct examination. Plating onto Malt Extract Agar (MEA), Sabouraud Dextrose Agar (SDA) and Rose Bengal Agar (RBA).
- Macro and micro morphological characters were studied. The isolates will be identified at species level through a polybasic approach was used.
- Cryopreservation at the culture collection of DISTAV (CoID-UNIGE), member of MIRRI-IT.

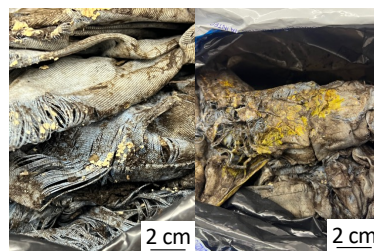


Figure 1. Shirt, front and back side.

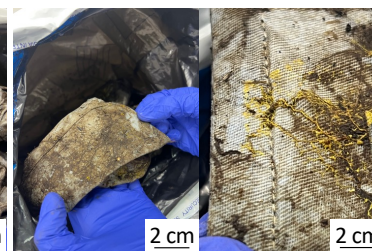


Figure 2. Shirt collar, different sides.

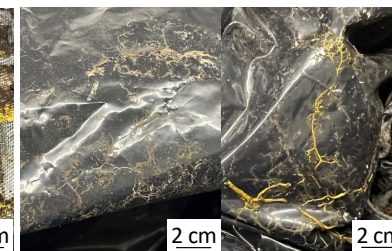


Figure 3. Plastic bag with mycelium.

Results

Micro fungi were sampled at different spot on the shirt, from each plate (Figure 4) different isolates were then purified. Different media and material selected different isolates, as shown in the Figure 4, reaching a total of 14 records.

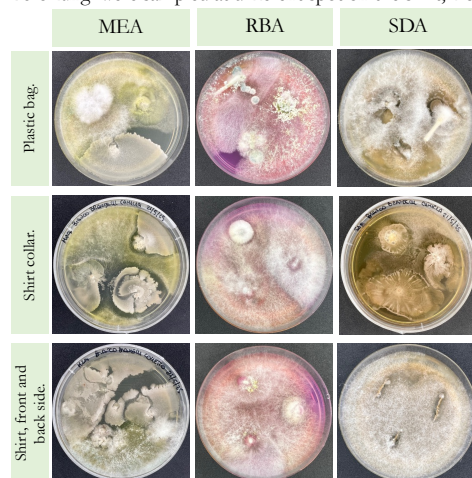


Figure 4. Petri dishes Ø 9cm with different media used for direct isolations.

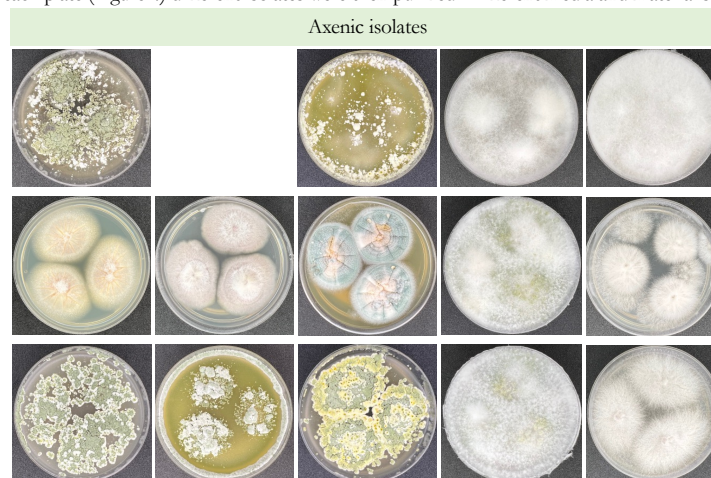


Figure 5. Subcultures on MEA in Petri dishes Ø 6cm.

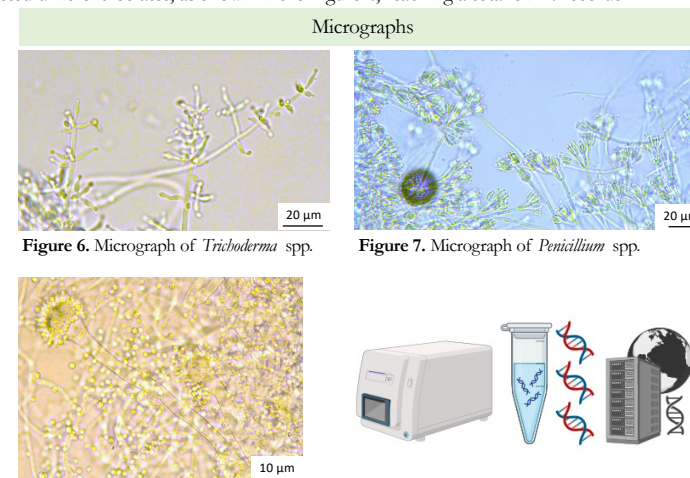


Figure 6. Micrograph of *Trichoderma* spp.

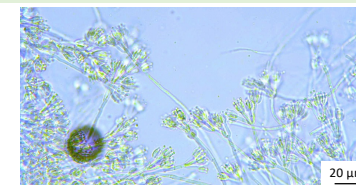


Figure 7. Micrograph of *Penicillium* spp.

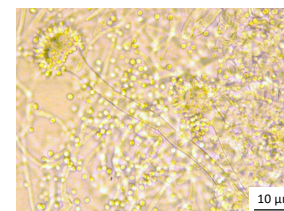


Figure 8. Micrograph of *Aspergillus* spp.

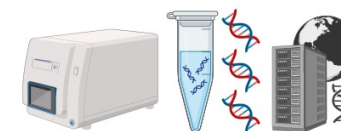


Figure 9. Work in progress.

Conclusions

Within the present research, 14 isolates have been identified through macro- and micro-morphological features and genetic studies are undergoing to confirm the identification of the strains. The outcome has contributed to the knowledge of the forensic science by characterize the presence, diversity and growth of fungi on forensic relevant materials, starting a Forensic Fungal Database, as well as improving the culture Collection of DISTAV (CoID-UNIGE), MIRRI-IT member, and project partner of the SUS-MIRRI.IT.

Acknowledgement and References

The present research has been funded by the European Union, Marie Skłodowska-Curie Actions, Project Natural Traces - Grant Agreement 101120165, for further details kindly consult: naturaltraces.com
Di Piazza, S., Zotti, M., Barranco, R., Cecchi, G., Greco, G., & Ventura, F. (2018). Post-mortem fungal colonization pattern during 6 weeks: Two case studies. *Forensic Science International*, 289, e18–e23.

<https://doi.org/10.1016/j.forsciint.2018.05.037>
Parmar, T. K., Rawtani, D., & Agrawal, Y. K. (2016). Bioindicators: The natural indicator of environmental pollution. *Frontiers in Life Science*, 9(2), 110–118. <https://doi.org/10.1080/21553769.2016.1162753>
<https://app.biorender.com/illustrations/>