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DIFFERENTIATION OF MYCOBACTERIUM ABSCESSUS COMPLEX BY FOURIER-TRANSFORM INFRARED SPECTROSCOPY

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Background

The *Mycobacteroides abscessus* complex (MABSC) is a group of rapidly growing, nontuberculous mycobacteria (NTM). It is commonly found in clinical settings, particularly in patients with compromised immune systems, such as those suffering from cystic fibrosis, HIV, COPD, and bronchiectasis. Treating MABSC is difficult due to its resistance to antibiotics, and the treatment outcomes are often disappointing.

MABSC has three subspecies: *M. abscessus* subsp. *abscessus* (MAA), subsp. *massiliense* (MAM), and subsp. *bolletii* (MAB). Each of these subspecies has unique clinical outcomes and specific profiles of susceptibility to antimicrobials. Therefore, it's essential to differentiate between these MABSC subspecies for effective patient treatment management. Currently, this differentiation can only be achieved using DNA-based techniques.

In this study, we explore the potential of **FT-IR spectroscopy (FTIRS)** for the **discrimination** between **MABSC subspecies**

Material and methods

- ✓ A total of N=41 MABSC strains, both patient-derived and culture collection strains were included in this study. The dataset includes n=18 MAA (11 smooth, 7 rough phenotype), n=13 MAM (11 smooth, 2 rough phenotype), and n=10 MAB (7 smooth, 3 rough phenotype).
- ✓ All strains were analysed by the FTIRS-based IR Biotyper® system (IRBT - Bruker Daltonics, Germany), following the manufacturers instruction (see **Figure 1**). Three independent biological replicates on Löwenstein-Jensen medium incubated for 72 h at 35±2 ° C were included.

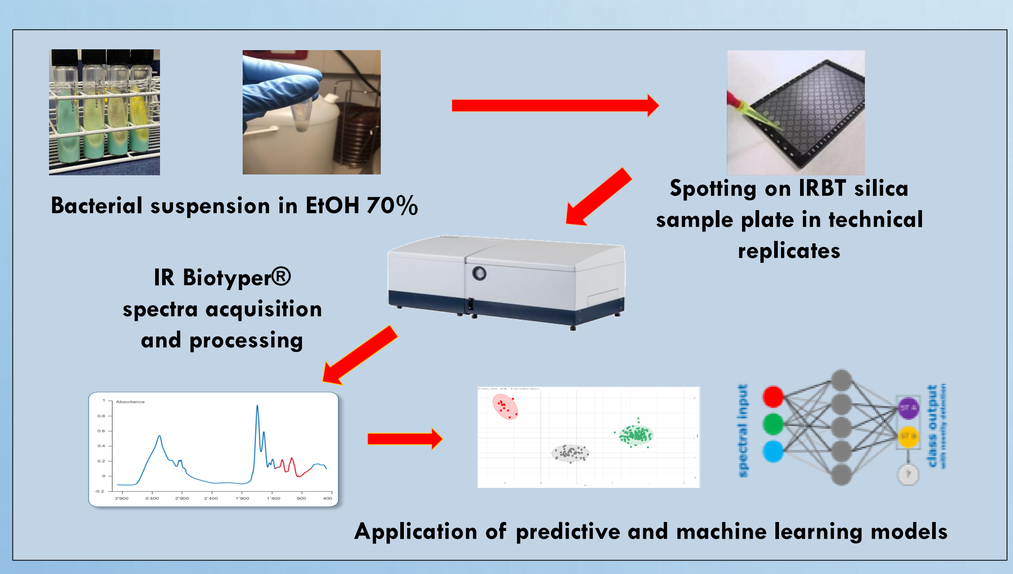

Figure 1. IR Biotyper® workflow

- ✓ **Spectra acquisition, processing and data analysis** were performed by the **IR Biotyper® software V4.0**. The resolution power using different IR absorption spectral regions was investigated.
- ✓ **Principal components analysis (PCA)** and **linear discriminant analysis (LDA)** were applied to the whole dataset of isolates for exploratory investigation of the discriminability of the three subspecies, and to create predictive models.

Results

The wavenumber range which allowed the best resolution was the one corresponding to the absorption of carbohydrates and lipids (1300-800 cm^{-1}, 1500-1400 cm^{-1} and 3000-2800 cm^{-1}).

PCA-LDA analysis showed that the biggest spectral variance is caused by the colony phenotype, being either rough or smooth (see **Figure 2**).

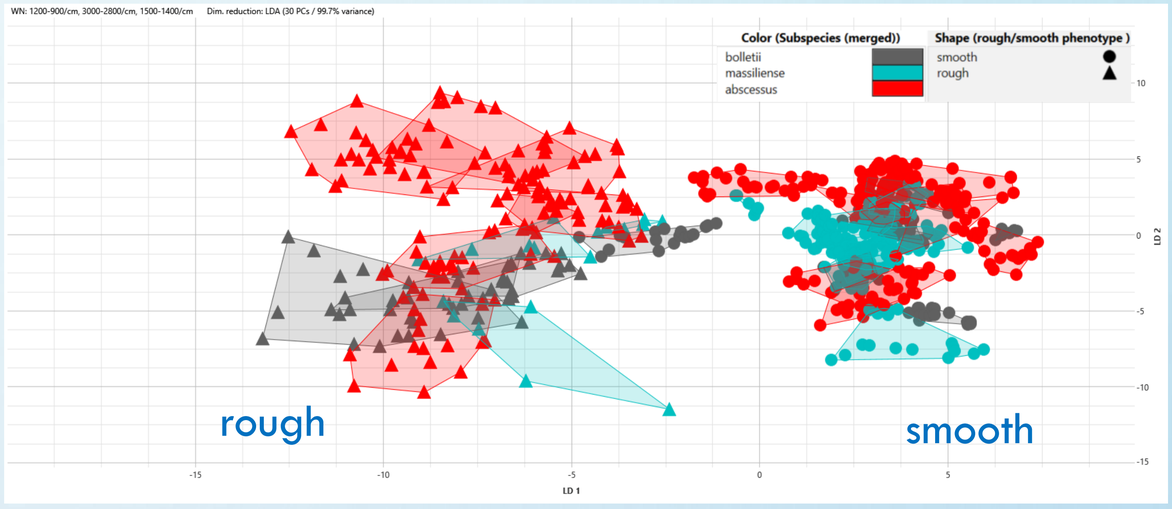

Figure 2. PCA-LDA clustering of the MABSC in relation to the colony phenotype. Each shape represents a spectrum; the convex hulls encompass spectra of the same isolate.

Considering the **smooth phenotype** group (total n=29 isolates), the LDA model, built with 18 isolates for the training and 10 isolates for the testing, shows a separation between the three subspecies (see **Figure 3**).

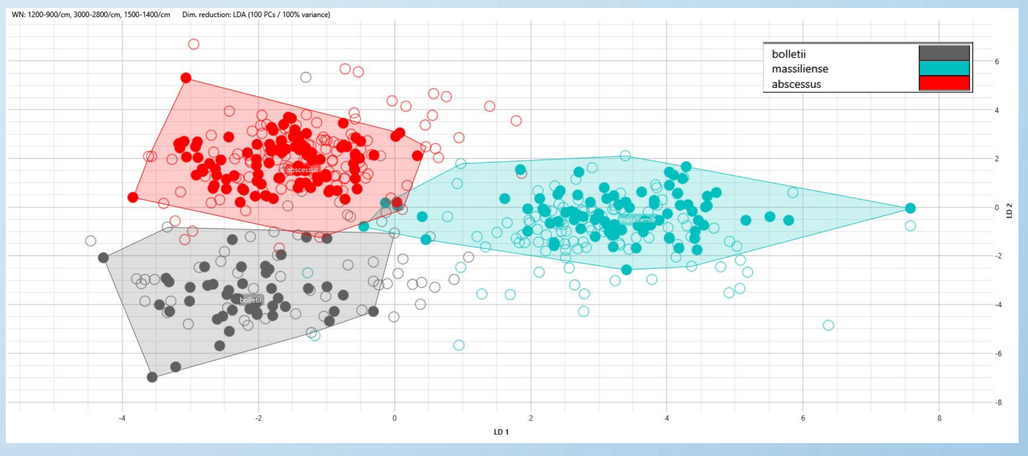

Figure 3. LDA predictive model for the MABSC smooth phenotype group. Filled shapes represent the spectra used for the training; empty ones represent testing spectra.

Considering the **rough phenotype** group (n=12 isolates), a robust LDA model could not have been built, because of the too low number of MAB and MAM. Nevertheless, exploratory data analysis showed the discriminability of the three subspecies (see **Figure 4**).

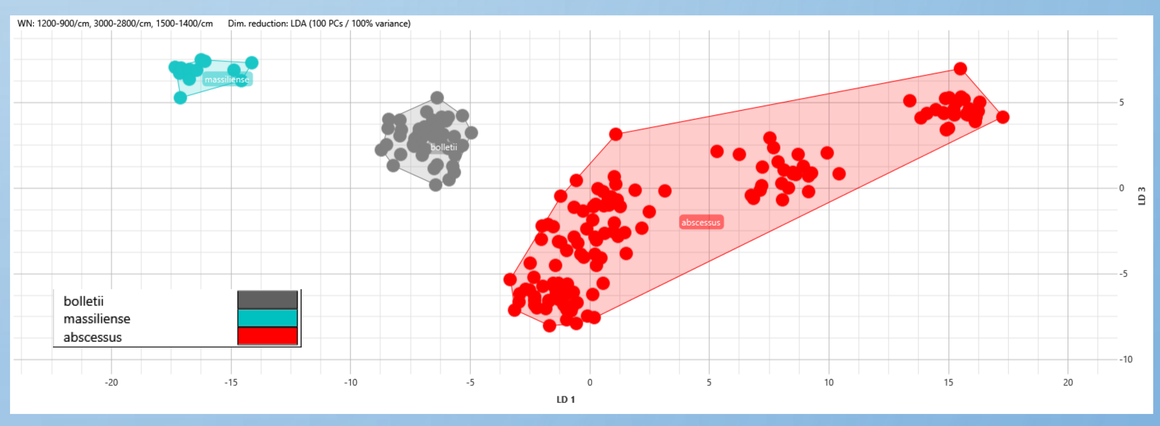

Figure 4. LDA clustering of the MABSC rough phenotype group.

Conclusion

The IR Biotyper demonstrated **encouraging outcomes in distinguishing within MABSC.** To truly understand the implications of this novel technology, we need more extensive studies with a significantly **larger sample size.** The goal is to **determine if** the **prediction models can be generalized to be universal.** The potential of machine learning should be explored with the objective of creating an **automated classification tool** using the IR Biotyper.

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