P0666

Application of Fourier-transform infrared spectroscopy for the differentiation of *Mycobacteroides abscessus* complex subspecies

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Background

Mycobacteroides abscessus complex (MABSC) belongs to the fast-growing nontuberculous mycobacteria (NTM) and is one of the most frequently isolated in clinical practice, especially in immunocompromised patients, such as those with cystic fibrosis, HIV-positive status, COPD, and bronchiectasis. Antimicrobial therapy of MABSC is challenging due to their antibiotic resistance and the outcome is often poor. The three subspecies of MABSC, namely M. *abscessus* subsp. *abscessus* (MAA), subsp. *massiliense* (MAM), and subsp. *bolletii* (MAB), exhibit distinct clinical outcomes and characteristic antimicrobial susceptibility profiles. Therefore, distinguishing between MABSC subspecies is crucial for accurate patient therapeutic management, but it can only be achieved through DNA-based methods at present.

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 analyzed by the FTIRS-based IR Biotyper® system (IRBT - Bruker Daltonics, Germany), following the manufacturers instruction (**Figure 1)**. Three independent biological replicates on Löwenstein-Jensen medium incubated for 72 h at 35±2 ° C were included.

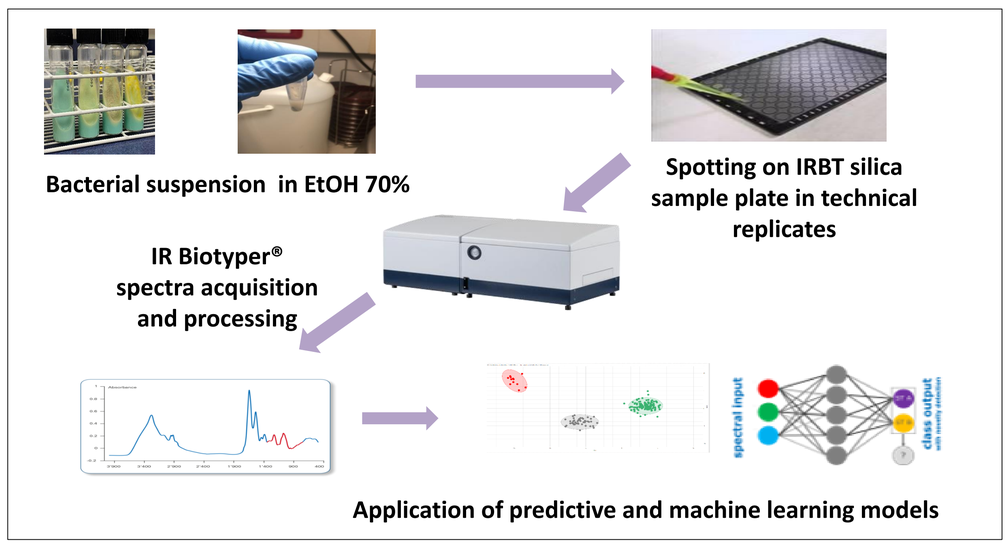

Figure 1. IR Biotyper® workflow

- ✓ **Spectra acquisition**, **processing** and **data analysis** were performed by the IR Biotyper® software V4.0. The spectral regions corresponding to the IR absorption by carbohydrates and lipids were used (1300-800 cm^{-1}, 3000-2800 cm^{-1}, and 1500-1400 cm^{-1}).
- ✓ **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.
- ✓ **Predictive LDA models** were developed, using random 70% of the isolates for the training, and the remaining 30% for the testing.

Results

Overall, the PCA-LDA score plot showed that the biggest spectral variance is caused by the colony's phenotype (rough or smooth) - **Figure 2**

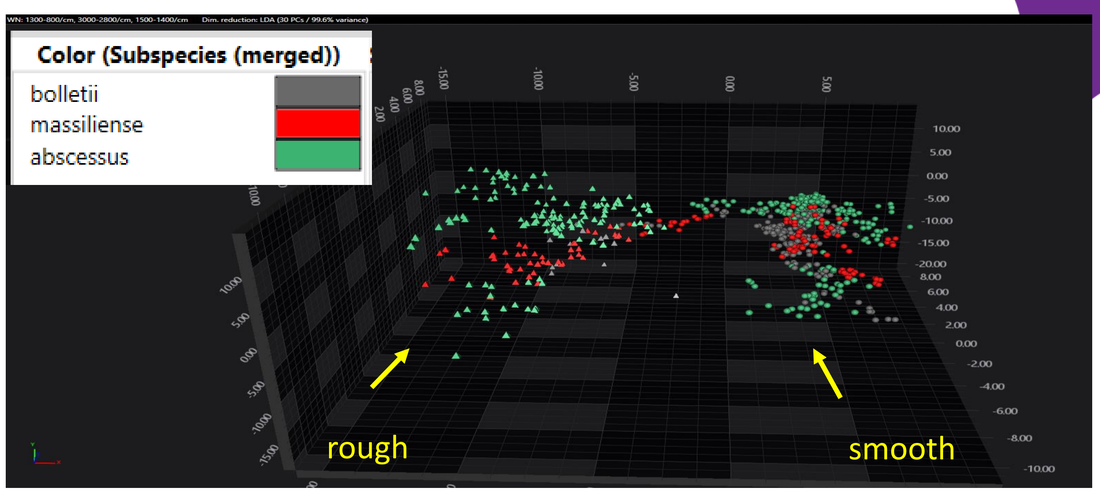

Figure 2. PCA-LDA clustering of the MABSC in relation to the colony phenotype.

Considering the **smooth phenotype** group (tot. 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 (**Figure 3**).

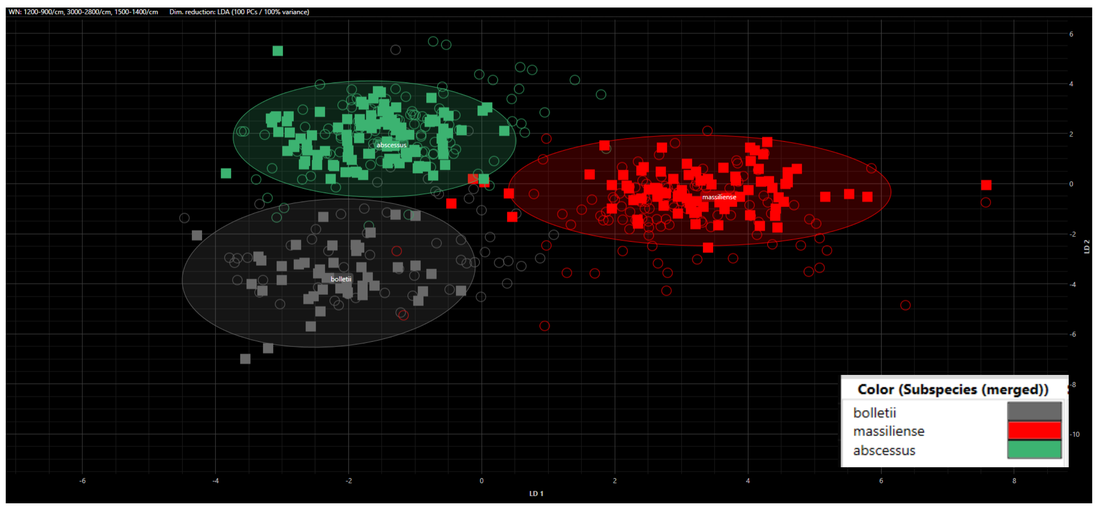

Figure 3. LDA predictive model for the MABSC smooth phenotype group. The filled geometric form represent the spectra used for the training; the empty ones represent the testing spectra. The ellipses correspond to the 95 CI.

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 (**Figure 4**).

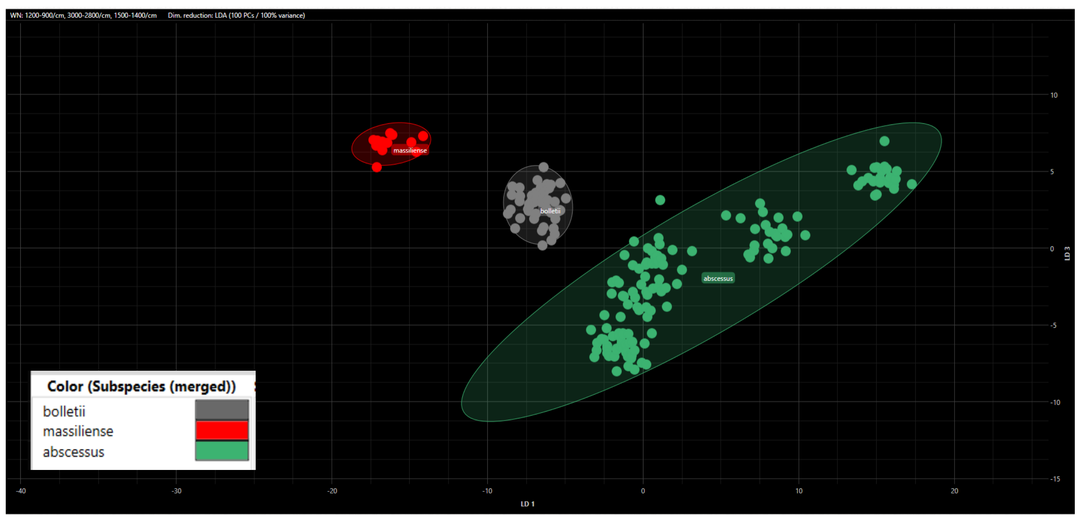

Figure 4. LDA clustering of the MABSC rough phenotype group.

Conclusion

IR Biotyper showed **promising results for the discrimination within MABSC**. Further studies with a much **larger number of samples** are needed to assess the real impact of this new technology's application, and to evaluate the possibility to **generalize the prediction models** to an "universal" extent. **Machine learning capabilities** should be investigated to aim to the development of an **automated classification tool** by IR Biotyper.

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34th ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
Barcelona, Spain
27 – 30 April 2024