P3723

Evaluation of WGS and FTIR spectroscopy for *Candida auris* clinical isolates typing

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Background

Candida auris (proposed as *Candidozyma auris*) is an emerging nosocomial pathogen, representing a global threat due to its significant mortality rate, resistance to antimycotics and disinfectants, and high potential of person-to-person transmission.

Global guidelines recommend an active surveillance for the rapid identification of colonization in high-risk patients.

WGS provides high resolution data SNPs (single-nucleotide polymorphisms) and allelic differences. Nevertheless, clear and universal typing schemes and thresholds to assess relatedness between strains are still to be defined, especially regarding cross-transmission, as mutation rates, host factors and bioinformatic tools play a role.

In this study, we compared Fourier Transform Infrared (FTIR) spectroscopy with Whole Genome Sequencing (WGS) for the typing of *C. auris*, investigating a dataset including clonally related, likely unrelated strains and unrelated strains.

Material and methods

Sample set

Overall, **n=71 *C. auris*** strains, belonging to clade I (n=57) and clade III (n=14) were included in this study. The strains were collected from 31 patients of Johns Hopkins Healthcare System (Baltimore, USA) between 2021 and 2024.

The isolates, identified at species level by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and previously typed by WGS (Illumina, San Diego, USA), were independently analyzed on the FTIR-based IR Biotyper® (IRBT) system (Bruker Daltonics, Germany).

FT-IR and data analysis

FT-IR analysis was performed by the IR Biotyper® system (IRBT - Bruker Daltonics, Germany), following the manufacturers instructions.

IR spectra were acquired from three independent cultures on Sabouraud dextrose agar (Becton and Dickinson, Germany) grown at 36 °C for 24±1 h, following the manufacturer's instructions.

Principal component analysis (PCA) and linear discriminant analysis (LDA) were used to determine the centroid distances between the isolates, reported in a distance matrix.

Comparison between genomic and infrared spectral distances were performed using a Mantel test [1,2] implemented in the PAST software version 5.22 [3].

The estimated significance (one-tailed p-value, Mantel test) was conducted using ~10^6 permutations, and the lowest attainable p-value in this instance is 1/(N+1).

Results

➢ IRBT PCA/LDA results show **clear discrimination of the two *C. auris* clades, and further subclustering** within the two clades (Figure 1).

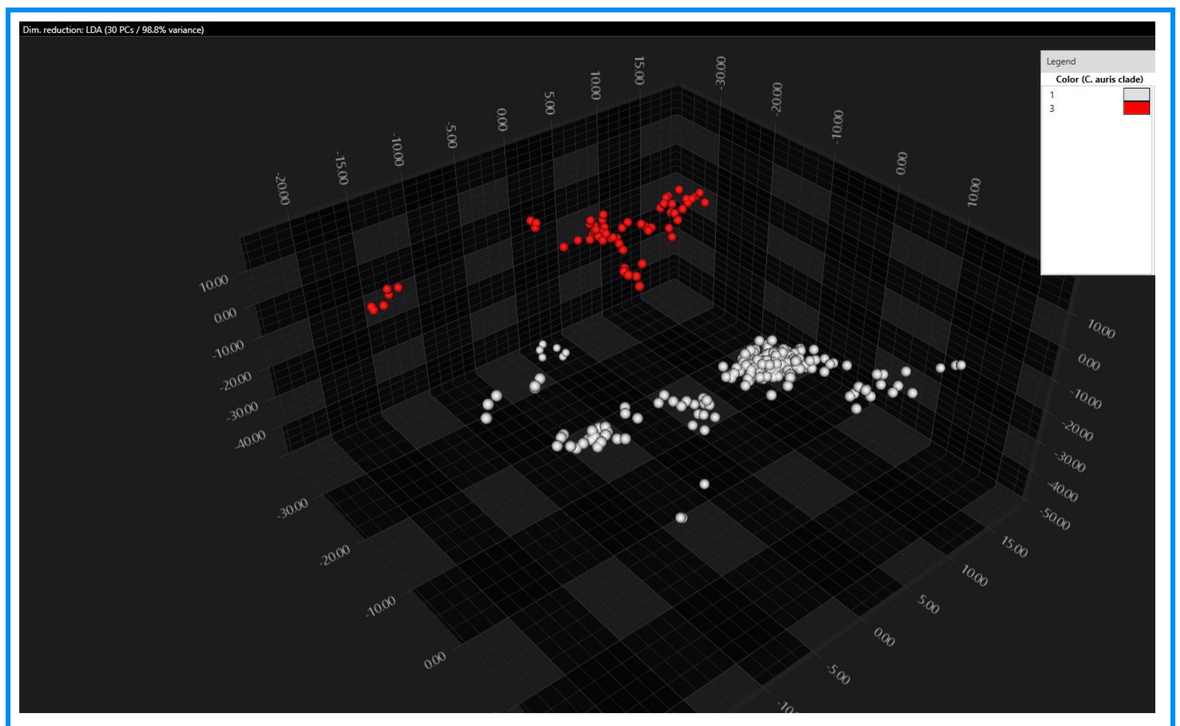


Figure 1. 3D LDA scatterplot showing differentiation of *C. auris* clade I and clade III in the spectral space. LDA was performed using 30 PCs, representing 97.2% of the spectral variance. Each clade is depicted in a different color. Each sphere represent a spectrum.

➢ The clades exhibited an intra-clade genetic distance between 0 and 35 (clade III) or 97 (clade I) alleles.

➢ **IRBT spectral distance was in very good correlation with genetic distance (R = 0.722, p < 0.0000001)** (Figure 2).

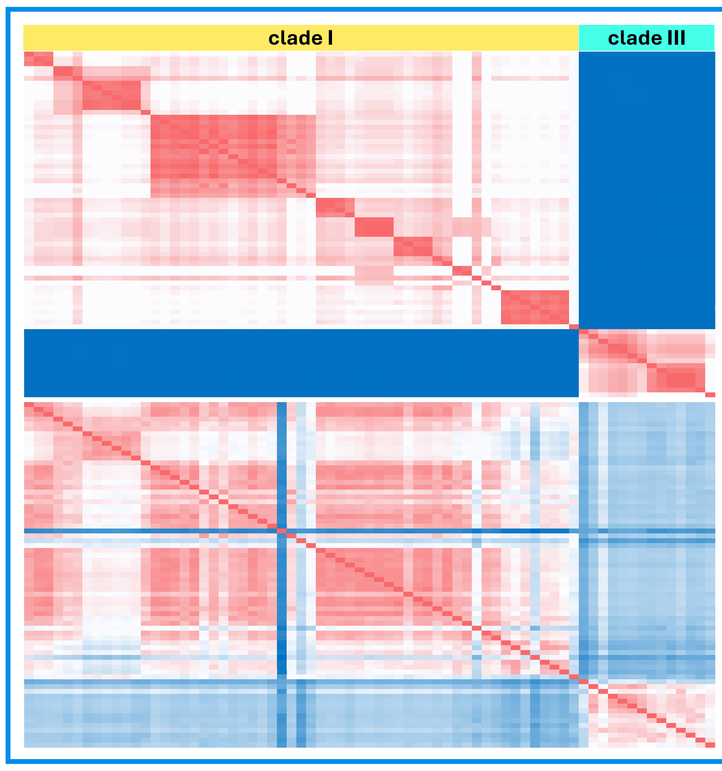
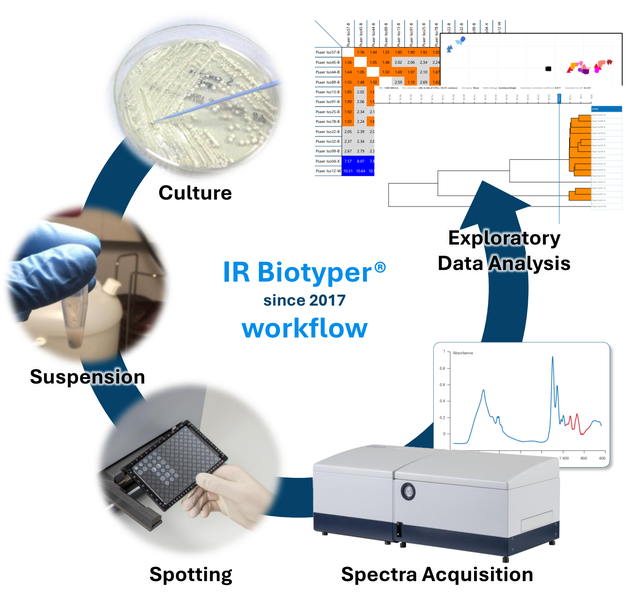


Figure 2. Genomic (top) and IR-spectral (bottom) distance matrices with identical isolate ordering. The genomic clustering was used as the reference for sorting.
Genomic distances range from 0 SNPs (red) to 3,454 SNPs (blue), with white marking the median of 30. Spectral distances, measured in arbitrary units, range from 0 (red) to 110 (blue), with white marking the median of 33.



References

[1] Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209-220.

[2] Mantel, N. & R.S. Valand 1970. A technique of nonparametric multivariate analysis. Biometrics 26:547-558.

[3] Hammer, Ø., Harper, D.A.T., Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1): 9pp.

Conclusion

IRBT showed a very good correlation with genomic data in terms of strains similarity. More data is needed with both methodologies to define clear and universal thresholds to assess the relatedness among different strains, especially in the grey-zone of genomic distance between the close/in-patient threshold (<13 SNPs) and the threshold proposed for surely unrelated strains (>50 SNPs).

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Not for use in clinical diagnostic procedures

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