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SUPSI

DISCRIMINATION OF CLOSELY RELATED POPULATIONS BY MALDI-TOF MASS SPECTROMETRY USING THE ABOS SOFTWARE: *TRICHOPHYTON RUBRUM SENSU LATO* AS A MODEL

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Introduction

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is increasingly employed for the identification of filamentous fungi but the available software does not always allow discriminating closely related or cryptic species.

The dermatophytes *Trichophyton rubrum sensu stricto*, *T. soudanense* (also called the "African" *T. rubrum*) and *T. violaceum* have been shown to be very closely related at the molecular level. The standard MALDI-TOF MS software was also not able to clearly discriminate these species.[1,2]

We therefore used a newly developed software (ABOS) to try to separate *T. rubrum sensu stricto* from the "African" *T. rubrum* and *T. violaceum* from the other two species.

Strains

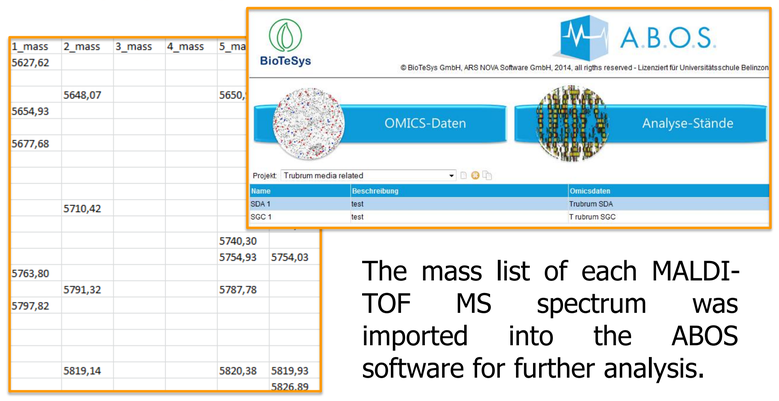
Strains were selected on the basis of their morphological identification (considered here as the gold standard). We used two culture media, i.e. Sabouraud Dextrose Agar with (SDA) or without (SGC2) gentamicin/chloramphenicol.

MALDI-TOF MS

An ethanol, 70% formic acid, acetonitrile protein extraction was carried out before standard MALDI-TOF MS analysis and identification (Vitek MS RUO, bioMérieux)[2].

		No. of mass spectra	
		SDA	SGC2
T. rubrum sensu stricto	R	100	94
"African" *T. rubrum*	A	64	33
T. violaceum	V	10	9
	TOTAL	**174**	**136**

Transfer to ABOS



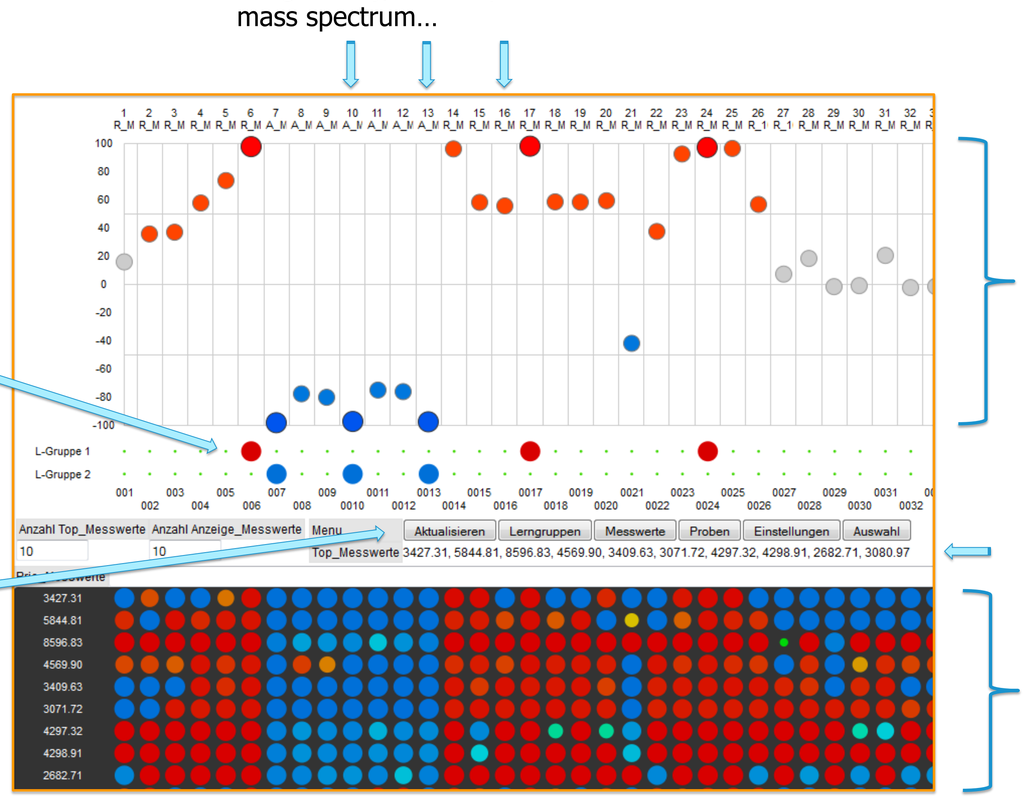
The mass list of each MALDI-TOF MS spectrum was imported into the ABOS software for further analysis.

ABOS procedure

1. ABOS compares 2 groups. For each group, 5 representatives (strains identified by ITS sequencing), called **references** are chosen. Here: red dots are ref. spectra of *T. rubrum sensu stricto* (R), blue dots are ref. spectra of the "African" *T. rubrum* (A).

2. Each change is validated. Using a combination of statistical and geometric methods ABOS compares all parameters of all sample data and calculates differences and similarities to the 5 R and 5 A references.

3. Each column (R and A = species assignment), corresponds to a mass spectrum…



4. … to which a colored point is assigned after validation:
- Red point: within certain thresholds, the mass spectrum shows similarities with the R group;
- Blue point: the mass spectrum shows similarities with the A group;
- Gray point: not enough similarities to allow assigning the mass spectrum to the R or A groups.

5. The 10 masses that are most significant to detect differences or similarities are shown.

6. The contribution of the 10 most significant masses to the decision process is indicated by the ranking (top = most relevant) and through the color and size of the dots (red = mass/peak appearance similar to R reference; blue = mass/peak appearance similar to A reference group).

Results

Morphology: gold standard.
MALDI-TOF MS standard identification: low discrimination identifications were considered as **doubtful**.
ABOS identification: all ABOS reference strains were identified by ITS sequencing; gray points were considered as **doubtful**; for this work, 5 different groups of reference spectra were randomly selected for the analysis and the average identification outcome (0: no identification at all; 1: perfect identification) of each group is presented.

Results were divided into: **all data** (**doubtful** data were **included**) and **only confirmed data** (**doubtful** data were **excluded**).

Comparison between:	Ratio of correctly identified mass spectra (scale: 0-1; see results)							
	All data				Only confirmed data			
	SDA		SGC2		SDA		SGC2	
	MALDI-TOF MS	ABOS	MALDI-TOF MS	ABOS	MALDI-TOF MS	ABOS	MALDI-TOF MS	ABOS
V and **R/A**	**0.603**	**0.540**	**0.537**	**0.450**	**0.795**	**0.875**	**0.664**	**0.807**
R and **A**	**0.573**	**0.759**	**0.732**	**0.757**	**0.752**	**0.955**	**0.894**	**0.900**

***T. rubrum sensu lato* (R/A) versus *T. violaceum* (V).**
All data: *T. rubrum sensu lato* and *T. violaceum* were not distinguished satisfactorily by both standard MALDI-TOF MS and ABOS software, but the first system was slightly better.
Only confirmed data: ABOS was more performant than standard MALDI-TOF MS.

***T. rubrum sensu stricto* (R) versus "African" *T. rubrum* (A).**
All ABOS results were better than standard MALDI-TOF MS and differences were more pronounced with SDA than with SGC2.

Discussion

When considering also doubtful identifications (**all data**), MALDI-TOF MS standard evaluation method was marginally superior to ABOS in differentiating between *T. rubrum sensu lato* and *T. violaceum*. On the other hand, ABOS was definitely more efficient in separating *T. rubrum sensu stricto* from the "African" *T. rubrum*.

In conclusion, both systems are only moderately effective in separating these three taxonomic groups.

There is an ongoing debate on how tenable the separation of the "African" *T. rubrum* from *T. rubrum sensu lato* can be.[3]

The results of this work cast some more doubts into this debate - is the "African" *T. rubrum* really a separate species or is it rather to be considered as a member of *T. rubrum sensu lato*?

Conclusions and outlook

ABOS shows promises of being very effective in separating MALDI-TOF MS data obtained from cryptic groups, although it may fail with groups as difficult as the *T. rubrum* complex. Work is presently ongoing in our lab to try to separate other cryptic species of filamentous fungi.

This system is very easy-to-use and results are readable and clear.
To our point of view, to render the ABOS software more attractive and powerful, it would be very nice to have the possibility to select more than two groups of reference samples. In the current status of the software a third reference group has to be examined by running a new, separate analysis.

References

1. De Respinis *et al*. 2013, *Med Mycol* 51: 514-521.
2. De Respinis *et al*. 2014, *J Clin Microbiol* 52: 4286-4292.
3. Gräser *et al.* 2008, *Mycopathologia* 166: 239-256