Identification of Aspergillus species using CE in capillary with roughened part and MALDI-TOF MS

Jiří Šalplachta[1], Anna Kubesová[1], Pavel Karásek[1], Filip Růžička[2], Michal Roth[1]

1) Institute of Analytical Chemistry of the ASCR, v. v. i., Veveří 97, 60200 Brno, Czech Republic
E-mail: salplachta@iach.cz

2) Department of Microbiology, Faculty of Medicine, Masaryk University and St. Anne's University Hospital, Brno, Pekařská 53, 65691 Brno, Czech Republic

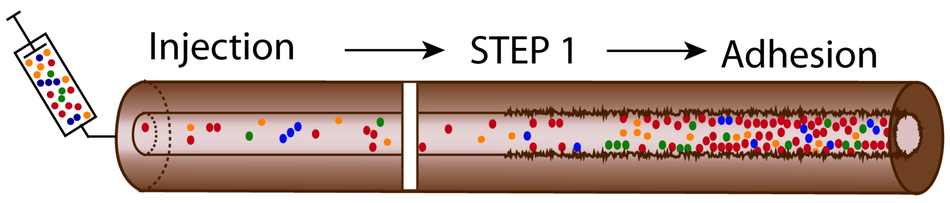
Introduction

Aspergillus is one of the most common and widely distributed genera of fungi. *Aspergillus* species produce airborne spores that are inhaled by humans which can lead to the infection particularly in immunocompromised patients. Timely and reliable identification of an etiological agent is therefore crucial for successful therapy. However, currently used diagnostic methods (radiological data, microbiological tools, and histopathologic examination) lack specificity and sensitivity and are time-consuming.

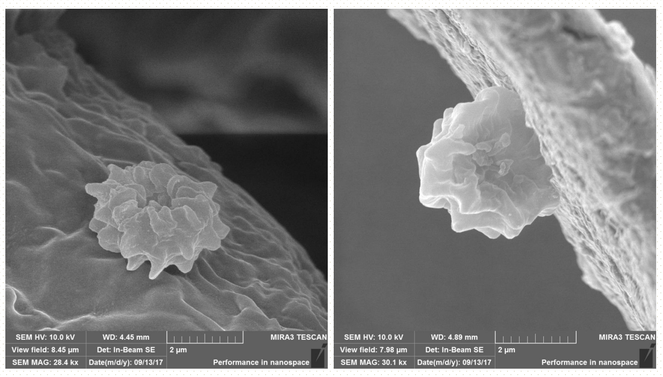
The goal of this study was to asses the ability of combination of capillary electrophoresis (CE) in SCW-etched FS capillary with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to identify *Aspergillus* spp. conidia in bronchoalveolar lavage fluid (BALF). Four *Aspergillus* spp., *A. niger*, *A. fumigatus*, *A. flavus*, and *A. parasiticus*, were selected as the most common causative agents of Aspergillosis. Since the lung is primary site of the infection, BALF was chosen as an optimal specimen for diagnosis of *Aspergillus* infection.

Results

1. Dynamic adhesion of *Aspergillus* conidia onto roughened part of FS capillary

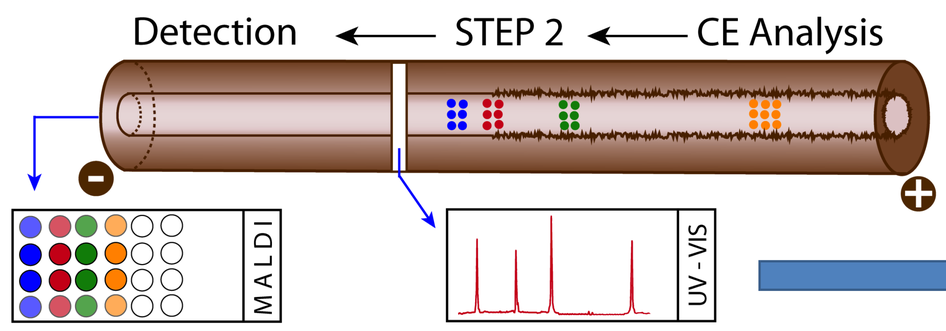


- total length of the FS capillary (100 µm ID, 360 µm OD): 500 mm; separation part: 350 mm from the anode; roughened part (etched with SCW): 250 mm from the anode
- sample: conidia of ***A. niger***, ***A. fumigatus***, ***A. flavus***, and ***A. parasiticus*** suspended in BALF sample (each of them 10^4 conidia mL^{-1}) with an additive of 1% PEG 400 (w/v)
- dynamic adhesion of 10 µl of the sample at a flow rate of 5 µL min^{-1}
- dynamic adhesion efficiency: 80%



Scaning electron microscopy of *A. niger* conidium adhered onto the inner wall of the FS capillary etched with SCW.

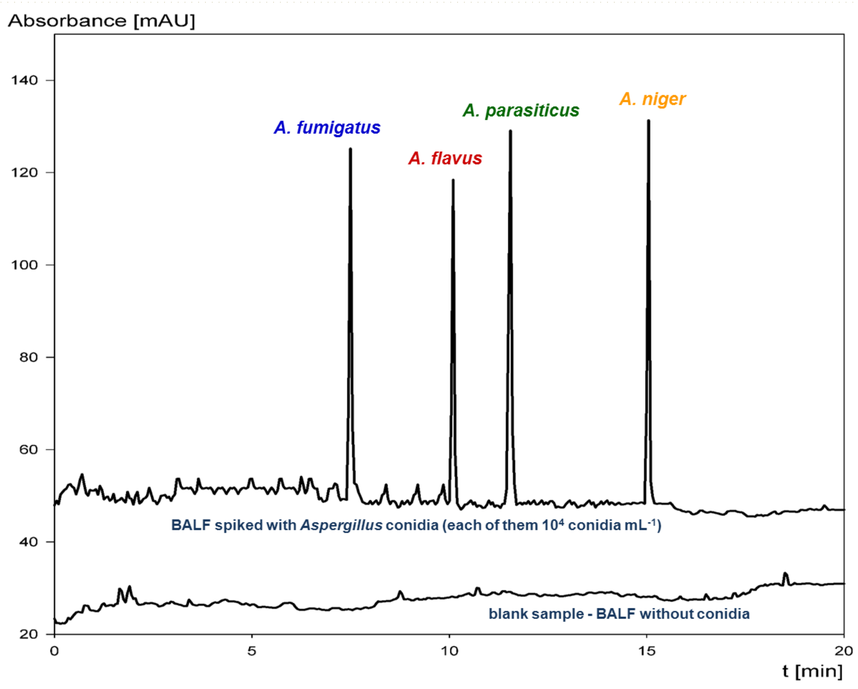
2. CE analysis and fraction collection



CE separation conditions

- BGE: 2×10^{-3} mol L^{-1} phosphate buffer, pH 7, together with dissolved additives, 10 % (v/v) EtOH, 0.3% (w/v) PEG 10 000, and 0.1 % (w/v) Brij 35
- separation at constant voltage -20 kV on the detector side
- UV detection at 280 nm

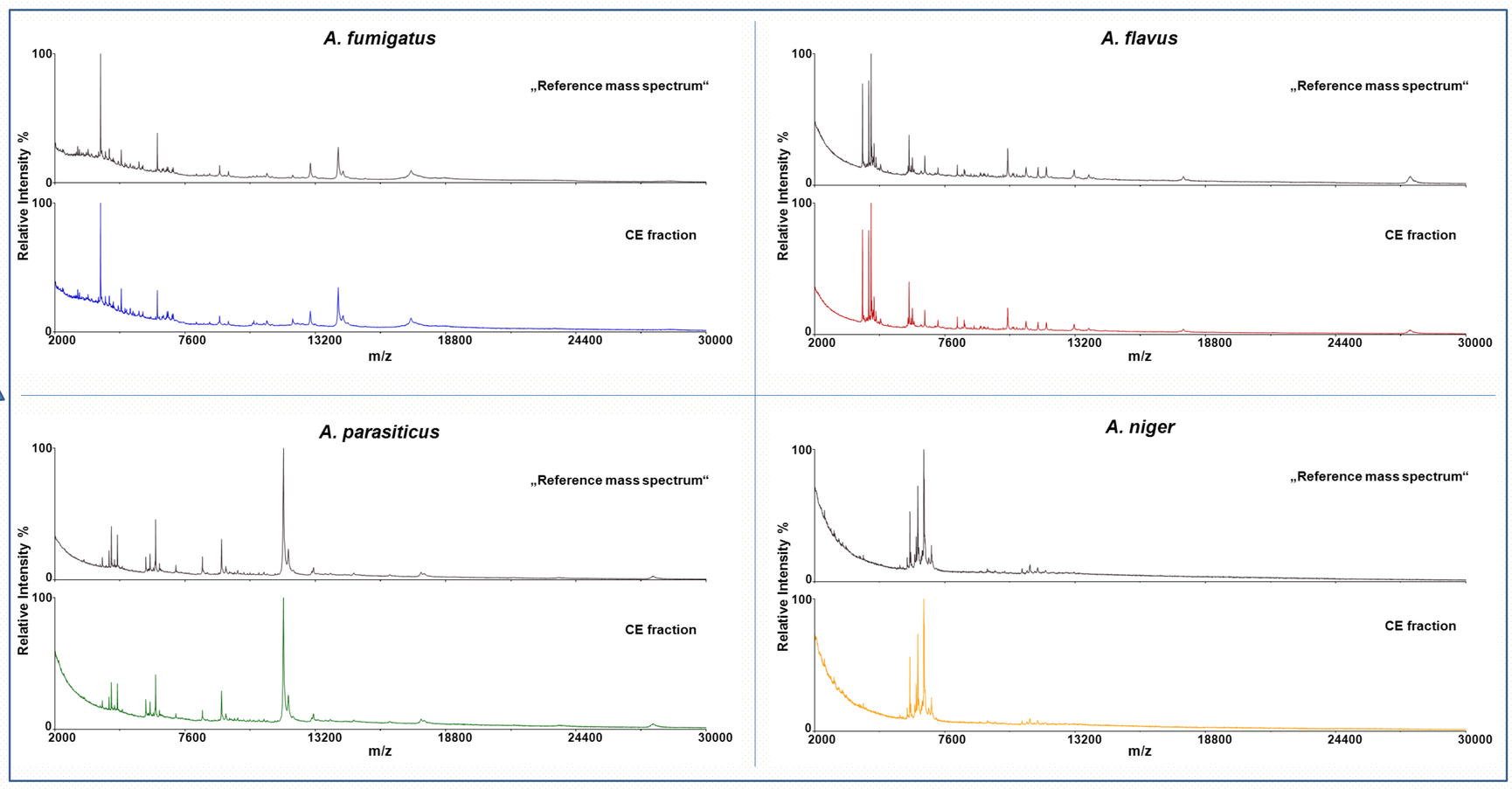
3. CE results



Fraction collection and deposition on MALDI sample plate

- once the first zone of conidia (*A. fumigatus*) had been detected, the analysis continued for additional 170 s when the zone was approximately 20 mm before the cathodic end of the capillary
- electrodes were displaced, a single-syringe infusion pump was immediately attached to the anodic end of the capillary and its content was pushed out of the capillary at a flow rate of 50 nL s^{-1}
- small drops (about 0.2 µL) were deposited manually every 4 s onto the MALDI plate (the droplet simply touched a sample spot of the plate previously overlaid with MALDI matrix)

4. MALDI-TOF MS results



MALDI-TOF MS

- all MALDI-TOF MS experiments performed on AB Sciex TOF/TOF 5800 System operating in linear positive ion mode
- sinapinic acid (SA, 20 mg mL^{-1} in ACN/2% TFA, 3:2, (v/v)) used as a matrix
- samples deposited on the MALDI plate using three-layer method: SA-sample-SA

„Reference mass spectra“

- conidia of the cultivated *Aspergillus* strains suspended in SA solution to the concentration 10^6 conidia mL^{-1}
- analysis of supernatant after centrifugation

Conclusions

The FS capillary with roughened part, prepared by etching with supercritical water, was successfully used for dynamic adhesion of *Aspergillus* conidia in BALF sample. Subsequently, the conidia were simply on-line pre-concentrated with the efficiency of 80% by the combination of transient isotachophoresis (tITP) stacking and sweeping and further separated by micellar electrokinetic chromatography (MEKC). The fractions of separated conidia were collected from the capillary and analyzed by MALDI-TOF MS. The MALDI results revealed that the conidia were completely separated from each other and from other sample components in MEKC.

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