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Original article

Detection of characteristic metabolites of *Aspergillus fumigatus* and *Candida* species using ion mobility spectrometry – metabolic profiling by volatile organic compounds

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Summary

Volatile metabolites of *Aspergillus fumigatus* and *Candida* species can be detected by gas chromatography/mass spectrometry (GC/MS). A multi-capillary column – ion mobility spectrometer (MCC-IMS) was used in this study to assess volatile organic compounds (VOCs) in the headspace above *A. fumigatus* and the four *Candida* species *Candida albicans*, *Candida parapsilosis*, *Candida glabrata* and *Candida tropicalis* in an innovative approach, validated for *A. fumigatus* and *C. albicans* by GC/MS analyses. For the detection of VOCs, a special stainless steel measurement chamber for the microbial cultures was used. The gas outlet was either attached to MCC-IMS or to adsorption tubes (Tenax GR) for GC/MS measurements. Isoamyl alcohol, cyclohexanone, 3-octanone and phenethylalcohol can be described as discriminating substances by means of GC/MS. With MCC-IMS, the results for 3-octanone and phenethylalcohol are concordant and additionally to GC/MS, ethanol and two further compounds (p_0642_1/p_683_1 and p_705_3) can be described. Isoamyl alcohol and cyclohexanone were not properly detectable with MCC-IMS. The major advantage of the MCC-IMS system is the feasibility of rapid analysis of complex gas mixtures without pre-concentration or preparation of samples and regardless of water vapour content in an online setup. Discrimination of fungi on genus level of the investigated germs by volatile metabolic profile and therefore detection of VOC is feasible. However, a further discrimination on species level for *Candida* species was not possible.

Key words: *Aspergillus fumigatus*, *Candida albicans*, *Candida* species, identification, metabolism.

Introduction

The prevalence of fungal infection is with about 20% of the treated infections on intensive care unit, a challenge for intensive care physicians.[1] Because of changes in treatment strategies and the increased use of antifungal prophylaxis, the epidemiology of invasive fungal diseases has changed substantially in recent years and infections caused by *Candida* species are no longer the majority in many institutions. In contrast, the emergence of non-*Candida* invasive fungal diseases such as aspergillosis and others has increased.[2] The treatment of suspected fungal infection represents a challenging dilemma for the clinician. Recently published guidelines recommend initial treatment of suspected invasive fungal disease with broad-spectrum antimycotic therapy.[3–5] The argument for this strategy is that several studies showed that immediate initiation of appropriate antimycotic therapy was associated with reduced mortality.[6–8] Considering the importance of appropriate initial antimycotic therapy in critically ill patients with

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invasive fungal infection, a de-escalating strategy (i.e., starting with broad-spectrum antimycotic therapy followed by an azole if appropriate) seems to be the preferred approach rather than starting narrow-spectrum therapy and then broadening the spectrum once culture data are available. Therefore, a timely diagnosis of microbiological origin of infection, based on a highly sensitive and specific assay, may reduce unnecessary broad-spectrum antimycotic therapy.

Different strains are classically determined using culture combined with biochemical or immunochemical identification and susceptibility tests. The major limitation of the current culture based technique is that results are not available early enough to guide antimycotic management.

To overcome the problems with pathogen identification in sufficient time, several investigations have been performed like a proteome-based identification of species by matrix-assisted laser desorption – time-of-flight mass spectrometry (MALDI-TOF). Moreover, yeast isolates can be identified down to species level.[9] The system offers a rapid method for routine identification of isolates in clinical microbiology.[10] PCR and galactomannan (GM) testing of bronchoalveolar lavage (BAL) fluid were initially reported to be highly sensitive for specific *Aspergillus* infections by some investigators, but subsequent studies have reported variable results.[11–16] However, standardisation and validation have not yet been attained for these platforms.[17]

Volatile organic compound (VOC) metabolites determined by gas chromatography/mass spectrometry (GC/MS) reflect microbial growth *in vitro*. As already known, microorganisms produce VOCs for various reasons like for inter- and intra species communication[18,19] to adjust the metabolism according to current conditions of their spatial environment like availability of oxygen. A novel approach for detection of VOCs in headspace of microbiological culture (volatile metabolom) is a multi-capillary column (MCC) equipped ion mobility spectrometer (MCC-IMS), which has also been used for the present investigation. Ion mobility spectrometry is a well established method for the detection of chemical warfare agents, explosives and illegal drugs.[20–23] Moreover, this technique has become highly interesting also for medical applications. In combination with a MCC for preseparation,[24–27] it is a suitable tool to analyse complex and humid gas samples. In addition IMS provides a high sensitivity (detection limits down to ng l^{-1}-range to pg l^{-1}-range or ppb_v-range to ppt_v-range, respectively) combined with high-speed data acquisition and relatively low technical expenditure.

Based on that fact, volatile metabolites of *Aspergillus fumigatus* and the four *Candida* species *Candida albicans*, *Candida parapsilosis*, *Candida glabrata* and *Candida tropicalis* have been analysed in this study by MCC-IMS in an innovative approach. In addition, VOCs of *A. fumigatus* and *C. albicans* were analysed by classical GC/MS for identification of unknown metabolites. Regarding the importance of early diagnosis and microbial identification, this study describes as a proof of principle the usage of MCC-IMS for identification of different fungal genera by determination of VOCs in the headspace above *A. fumigatus* and *Candida* species.

Methods

Microbial strains

For the determination of volatile metabolites from microbes, classified ATCC/DSM strains, purchased from DSMZ (Braunschweig, Germany), have been used. The microbes used in the study have been stored at −80 °C in Lysogeny Broth (LB-medium) supplemented with glycerol (25% v/v). The strains *Aspergillus fumigatus* (DSM 21023), *Candida albicans* (ATCC 5703), *Candida parapsilosis* (DSM 24508), *Candida glabrata* (DSM 24506) and *Candida tropicalis* (DSM 24507) have been investigated. *Aspergillus fumigatus* and *Candida* spp. were grown on Columbia sheep blood agar (Ref. 43041; BioMérieux, Nürtingen, Germany) for 24 h at 37 °C.

Sampling

For detection of VOCs of microorganisms, a special measurement chamber was constructed (ISAS, Dortmund, Germany), consistent of stainless steel, thermally controlled (37 °C), and equipped with a gas inlet and a gas outlet. Air (scientific quality; AIR LIQUIDE Deutschland GmbH, Düsseldorf, Germany) was applied to the chamber with 100 ml min^{-1} via the gas inlet. The gas outlet was attached to the MCC-IMS measurement system or to an adsorption tube for later GC/MS measurements by polytetraflouroethylene tubing (Bohlender GmbH, Grünsfeld, Germany). To describe the compounds related to media, a blank Columbia blood agar disc was measured at first. After blank measurement, media with microbial colonies (after 24 h incubation) were introduced into measurement chamber and a sample for MCC-IMS analysis was drawn after 5 and 25 min, respectively. In addition to MCC-IMS analysis, a sample for GC/MS measurement from headspace of *A. fumigatus* and *C. albicans* was achieved by adsorption of volatile microbial metabolites from 2 l

(100 ml min^{-1}) headspace air volume of microbial cultures on thermal desorption tubes filled with Tenax™ GR (GERSTEL GmbH & Co. KG, Mühlheim, Germany). Measurements have been repeated six times for each strain.

Multi-capillary column – ion mobility spectrometry

A custom designed MCC-IMS[28] with a β-radiation source (^{63}Ni, 550 MBq) for ionisation was used for analyses of volatile metabolites of microorganisms. As the operating principle of MCC-IMS has been described before,[20,29] only a brief description will be given here.

The technique of MCC-IMS is based on preseparation of a gas sample by MCC, ionisation of analytes and measurement of the drift time-of ions in synthetic air (scientific quality; AIR LIQUIDE Deutschland GmbH) at ambient pressure under influence of an external electric field.[20]

The gas chromatographic preseparation was performed isothermally at 40 °C on a 20 cm non-polar multi-capillary chromatographic column (OV-5; MULTICHROM, Novosibirsk, Russia). The column contains approximately 1000 capillaries with an inner diameter of 43 μm each and a film thickness of 0.2 μm. The sum of the single capillaries allowed a gas flow of 150 ml min^{-1}. All experimental parameters of the IMS are summarised in Table 1.

Table 1 Experimental parameters of the ^{63}Ni-ion mobility spectrometer (IMS).

Preseparation	Multi-capillary column OV-5, 20 cm operated at 40 °C constant T
Ionisation source	β-radiation (^{63}Ni), 550 MBq
Drift distance	12 cm
Electric field strength	330 V cm^{-1}
Shutter opening time	300 μs
Drift and carrier gas	Synthetic air
Drift gas flow	100 ml min^{-1}
Carrier gas flow	150 ml min^{-1}
Temperature	Ambient
Pressure	Ambient

In the following, eluting gas-phase analytes are ionised by a β-radiation source through charge transfer from reactant ions (Fig. 1). A Bradbury–Nielsen grid is opened every 100 ms for 300 μs, thus an ion cloud is introduced into the drift tube. Under the influence of an external electrical field, the ions move towards a detector (Faraday plate). During their drift to the detector, the ions collide with the drift gas molecules moving in the opposite direction. Thereby, ions are decelerated depending on their size and shape resulting in a specific constant resulting velocity and therefore they are in the totally separated ideal case. The ion drift time is measured and the velocity of the ions can be calculated for a known drift distance. Normalising drift velocity to the electric field leads to the so-called ion mobility. Further normalisation to temperature and pressure obtains the reduced ion mobility, which is characteristic for the particular ions and independent on the platform and on environmental conditions.[30]

Thermal desorption – gas chromatography/mass spectrometry (TD-GC/MS)

The VOCs were thermally desorbed from Tenax material using the following temperature programme: initial temperature of 25 °C was increased by 30 °C s^{-1} to 250 °C, 2.5 min isothermal at 250 °C, splitless for 2 min at a flow rate of 60 ml min^{-1}, using helium as a carrier gas. Volatile analytes were then transferred at 300 °C to an integrated cooled injection system (CIS;

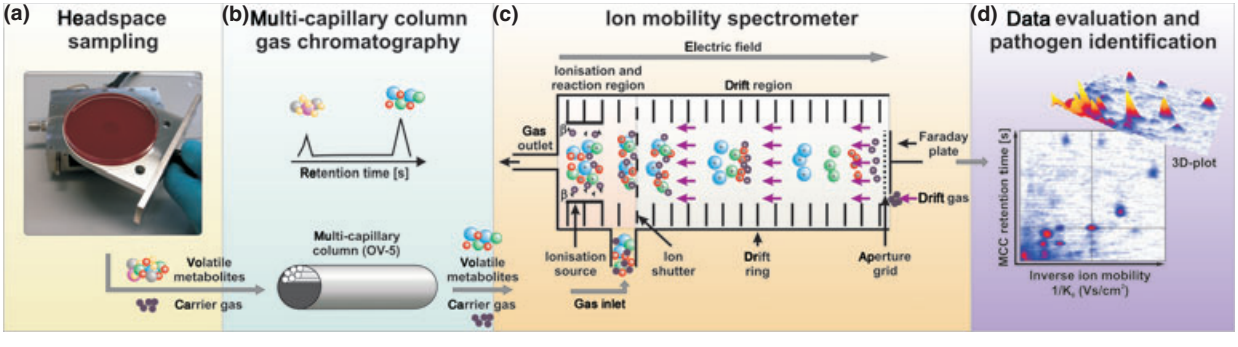

Figure 1 Scheme of the MCC-IMS used the detection of VOCs from microbial cultures. (a) Temperature and flow controlled sampling unit. (b) Separation of volatile metabolites on a multi-capillary GC column (MCC). (c) Ionisation and separation of charged analyte ions by their mobility. (d) Data evaluation.

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GERSTEL GmbH & Co. KG), focused at −120 °C, released onto a HP-5MS capillary column (60 m × 0.25 mm × 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA) after the CIS was heated to 250 °C (by 12 °C min^{-1}) and temperature was held for 10 min. The GC/MS analysis was performed on an Agilent Technologies 7890A GC system connected with an Agilent Technologies 5975C inert XL mass selective detector (MSD; Agilent Technologies) at an initial oven temperature of 35 °C, which was kept for 2 min, increased by 7 °C min^{-1} to 250 °C and held for 7 min. The separation was performed with helium as a carrier gas at a constant flow rate of 1.0 ml min^{-1}. Electron ionisation mode was set at 70 eV and the mass range of m/z 33–450 was measured.

MCC-IMS data analysis

Obtained MCC-IMS data have been evaluated using BB_IMSAnalyse 1.0 (ISAS, Dortmund, Germany). The resulting topographical plots (Fig. 2) represent the inverse ion mobility in [$Vscm^{-2}$] indicated by the x-axis and the retention time in seconds indicated by the y-axis. To correct minimal variations in retention time and in drift time, the data have been aligned by the before mentioned software.[30,31] The position of a signal in the topographical plot is characteristic and can be used to identify the corresponding metabolite by comparing with a database unknown detected compounds have been named after their position in the 2D-topographical plot as p_1000 x inverse ion mobility-

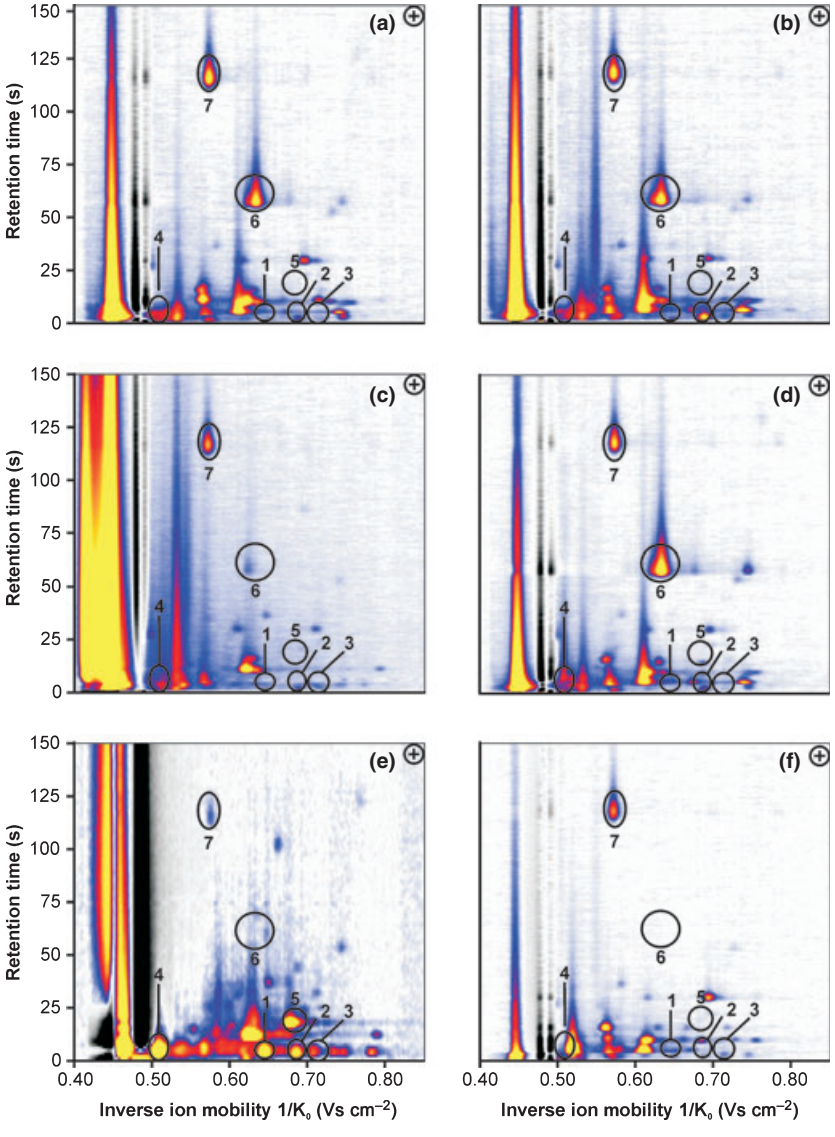

Figure 2 MCC-IMS headspace analyses of (a) *Candida albicans*, (b) *C. glabrata*, (c) *C. parapsilosos*, (d) *C. tropicalis*, (e) *Aspergillus fumigatus*, (f) Columbia blood agar. Peak heights/intensities are indicated by different colours (white = zero, blue = low, red = medium, yellow = high). The signals used for further analyses are 1: p_642_1; 2: p_683_1; 3: p_705_3; 4: ethanol; 5: 3-octanone; 6: phenethyl alcohol. Signal 7 represents benzothiazole, a contaminant of synthetic air which was used for an alignment of the retention time.

retention time (e.g. p_642_1). The signal intensity is a measure for the concentration of the substances.

GC/MS data analysis

After unambiguous identification of compounds by mass spectral analysis integrated signals either from selected fragment ion or total ion chromatograms (TIC; Fig. 3) have been used for data evaluation and comparison by AMDIS/NIST (Automated Mass Spectral Deconvolution and Identification System, version 2.62, 2005; NIST version 2.0, 2005, National Institute of Standards and Technology, Gaithersburg, MD, USA). For isoamyl alcohol *m/z* 55 (Rt = 8.5 min), for cyclohexanone *m/z* 55 (Rt = 12.5 min), for 3-octanone *m/z* 43 and 57 (Rt = 14.6 min) and for phenethyl alcohol *m/z* 91 (Rt = 17.7 min) have been analysed, respectively. We forbore the comparison of retention times measured with GC/MS with a native standard of the mentioned substances as described by Filipiak *et al* [32].

Statistical analysis

For descriptive statistics, values are presented as median (minimum–maximum).

Results

Volatile metabolites of *Aspergillus fumigatus* and *Candida* spp.

Both fungal genera examined gave different profiles of VOCs by MCC-IMS and TD-GC/MS (Figs 4 and 5).

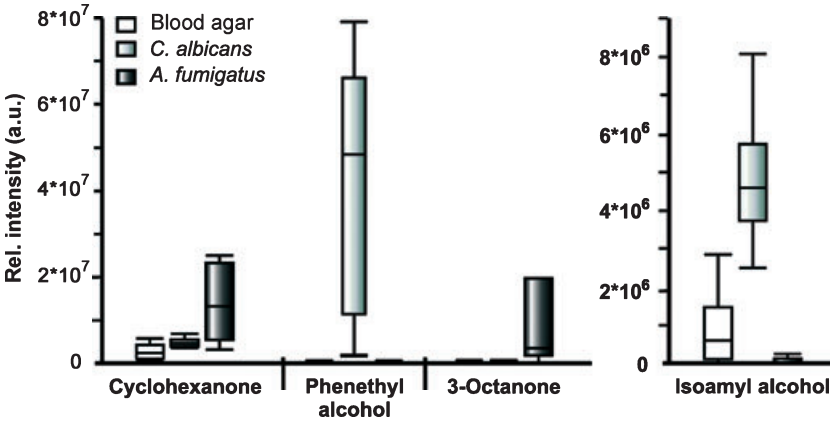

Figure 3 Box and whisker plot of volatile metabolites of *Aspergillus fumigatus* and *Candida albicans* determined by gas chromatography/mass spectrometry (GC/MS) (six measurements each). Box and whisker-plots with boxes indicating the 25%- and 75%-quantile, whiskers the 10%- and 90%-quantile and lines marking the median.

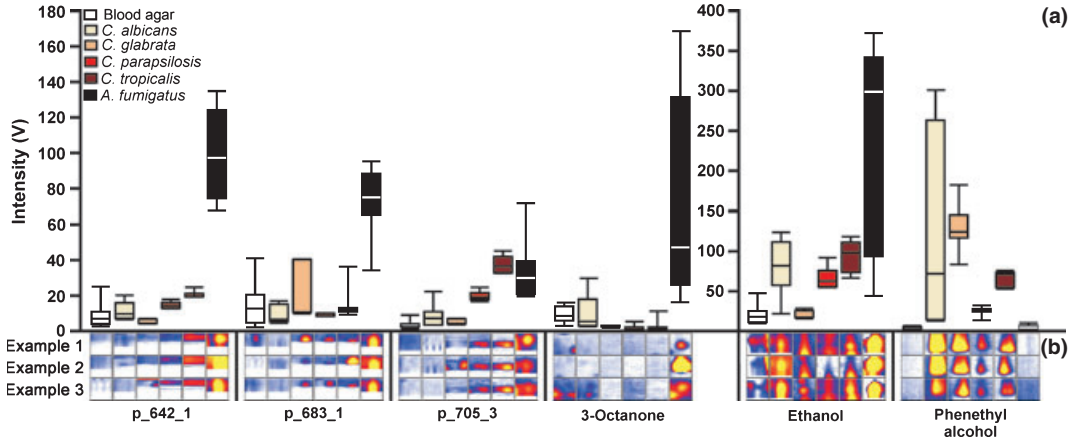

Figure 4 (a) Heatmap cut-out of three characteristic signals in three different exemplary samples determined with MCC-IMS for blood agar (top, white bars), *Candida albicans* (centre, grey bars) and *Aspergillus fumigatus* (bottom, dark bars). Three representative results are shown. (b) Box and whisker plot of volatile metabolites determined by MCC-IMS of *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *A. fumigatus* in comparison with Columbia sheep blood agar control (12 measurements each). Box and whisker plots with boxes indicating the 25% and 75% quantile, whiskers the 10% and 90% quantile and lines marking the median.

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Six volatile compounds were identified as characteristic for *A. fumigatus* by MCC-IMS and/or GC/MS (3-octanone, isoamyl alcohol, ethanol, cyclohexanone as well as so far unknown compounds p_642_1/p_683_1 and p_705_3). For *Candida* spp., four volatile compounds were found to be characteristic by either MCC-IMS and/or GC/MS (phenethyl alcohol, isoamyl alcohol, cyclohexanone and ethanol). Different metabolic profiles for the investigated *Candida* species cannot be described.

MCC-IMS analyses of *Aspergillus fumigatus* and *Candida* spp.

Five volatile compounds have been determined showing significant differences by MCC-IMS analysis in headspace concentrations above examined cultures (Fig. 4, Table 2). For the substances p_642_1 (and dimer p_683_1), p_705_3, ethanol and 3-octanone elevated levels for *A. fumigatus* compared with *Candida* spp. and blood agar control were measured for the substances p_642_1 (and dimer p_683_1), p_705_3, ethanol and 3-octanone by MCC-IMS, respectively. Substances in lower concentrations are observed as proton-bound monomers in MCC-IMS-plot. Beside proton-bound monomers, the formations of dimers or even trimers have been described for IMS previously.[33] Amongst others the formation of such multimers has been specified to depend on physicochemical properties, the concentration of a compound and experimental design. Furthermore, a dimerisation can be anticipated for various compounds when ^{63}Ni was used as ionisation source.[33] Elevated levels of phenethyl alcohol were measured with elevated levels in headspace of *Candida* spp. in comparison to *A. fumigatus* and blood agar. Isoamyl alcohol (3-methyl-1-butanol) and cyclohexanone each were under applied measurement conditions not detectable by means of MCC-IMS caused by co-elution with other substances. For unambiguous identification of analytes, MCC-IMS reference measurements for the substances ethanol (CAS 64-17-5), 3-octanone (CAS 106-68-3) and phenethyl alcohol (CAS 60-12-8) were executed under application of a calibration gas generator (HovaCAL)[34] separately.

GC/MS analyses of *Aspergillus fumigatus* and *Candida albicans*

By additional GC/MS analysis, enabling a definite identification of analytes, four volatile compounds could be identified as characteristic for *A. fumigatus* and *C. albicans* (Fig. 5, Table 3). For *A. fumigatus* cyclohexanone, and 3-octanone occurred in high amounts. Isoamyl alcohol and phenethyl alcohol were major

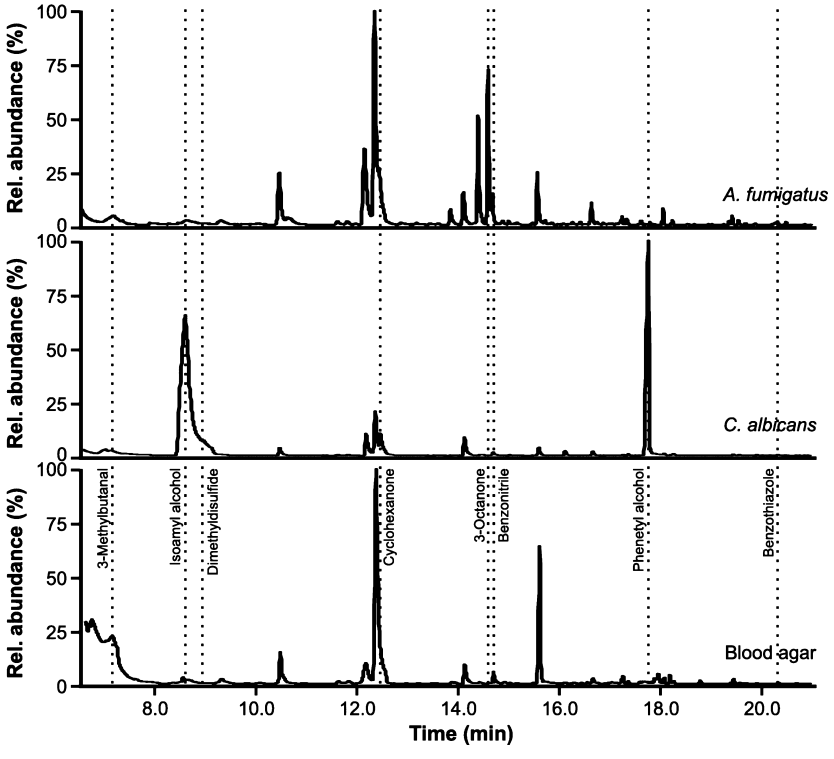

Figure 5 Gas chromatography/mass spectrometry (GC/MS) chromatogram of volatile metabolites formed by *Aspergillus fumigatus* and *Candida albicans* grown for 24 h on Columbia sheep blood agar and of the blank agar. Identified marker substances are highlighted grey.

Table 2 Multi-capillary column – ion mobility spectrometer (MCC-IMS) analyses of headspace of Columbia blood agar, *Candida* species and *Aspergillus fumigatus* [12 measurements each, signal intensity (mV), median (minimum–maximum)].

	Columbia blood agar	*Candida albicans*	*Candida glabrata*	*Candida parapsilosis*	*Candida tropicalis*	*Aspergillus fumigatus*
p_642_1	8 (3–25)	10 (6–20)	5 (4–7)	14 (13–18)	20 (19–25)	97 (19–156)
p_683_1	14 (2–41)	8 (5–17)	11 (10–41)	9 (9–10)	13 (10–36)	76 (29–121)
p_705_3	3 (1–9)	8 (4–22)	5 (4–7)	20 (17–25)	37 (33–45)	30 (8–120)
Ethanol	19 (10–47)	83 (22–123)	21 (15–28)	63 (54–92)	99 (66–118)	301 (41–380)
Isoamyl alcohol (3-methyl-1-butanol)	Not detected					
Cyclohexanone	Not detected					
3-Octanone	9 (3–16)	6 (3–30)	3 (2–4)	2 (1–6)	2 (1–11)	48 (15–322)
Phenethylalcohol	3 (1–6)	75 (12–295)	123 (82–179)	25 (13–32)	71 (52–74)	4 (1–12)

Table 3 Gas chromatography/mass spectrometry (GC/MS) analyses of headspace of Columbia blood agar, *Candida albicans* and *Aspergillus fumigatus* [six measurements each, relative intensity (a.u./10000), median (minimum–maximum)].

	Columbia blood agar	*Candida albicans*	*Aspergillus fumigatus*
p_642_1	Not detected		
p_683_1	Not detected		
p_705_3	Not detected		
Ethanol	Not detected		
Isoamyl alcohol (3-methyl-1-butanol)	59 (0–284)	465 (247–808)	0 (0–22)
Cyclohexanone	215 (48–545)	442 (324–654)	1308 (286–2465)
3-Octanone	0 (0–0)	0 (0–0)	318 (1–5386)
Phenethylalcohol	1 (0–36)	4888 (164–7932)	5 (0–22)

compounds in the headspace of *C. albicans*. Minor amounts of cyclohexanone could also be determined above *C. albicans* cultures.

Discussion

The aim of the study was to investigate if the detection of the volatile metabolic profile of four *Candida* species and *A. fumigatus* by the novel method MCC-IMS is possible and if the metabolic profile enables a differentiation. In a proof-of-principle setup with five characterised strains it could be demonstrated, that *A. fumigatus* strain DSM 21023 and the *Candida* species grown on Columbia blood agar can be differentiated by their metabolic profile. However, the described metabolic profile of the investigated *Candida* strains *C. albicans* (strain ATCC 5703), *C. parapsilosis* (strain DSM 24508), *C. tropicalis* (strain DSM 24507) and *C. glabrata* (strain DSM 24506) allowed no differentiation on species level. Differences in volatile metabolic compounds on genus level can be detected with both, the standard TD-GC/MS and the IMS equipped with MCC after an incubation of 24 h prior to measurement.

The two most abundant volatile compounds of *Candida* spp. in the experimental setup with cultivation on Columbia blood agar were isoamyl alcohol and phenethyl alcohol. Unspecific compounds with higher volatile concentrations over cultivated *A. fumigatus* and *Candida* spp. compared with blood agar were ethanol and cyclohexanone. Isoamyl alcohol was detected with GC/MS over *C. albicans* in significantly higher levels than over Columbia blood agar and was only in traces detectable over *A. fumigatus*. For unequivocally identification of compounds with MCC-IMS, the determination of a specific retention time and drift time is essential. If two or more compounds elute at a similar retention and drift time, a superposition of these substances is possible and will make the identification of these compounds difficult or even impossible. Therefore, the compounds isoamyl alcohol and cyclohexanone could not be separated properly with the used experimental setup for MCC-IMS.

The specific metabolic profile of *A. fumigatus* was defined by 3-octanone, p_642_1/p_683_1 and p_705_3. The compounds p_642_1/p_683_1 and p_705_3 eluted in seconds from MCC. Estimated GC retention times of these compounds are supposed to be below 3 min from the used GC column (HP-5MS, 60 m).[35] In this range of retention time in GC/MS mainly substances with low molecular weight are expected. A disambiguate identification of these compounds by GC/MS with the applied GC/MS setting was not possible as mass detection below m/z 33 were omitted to avoid disturbances from oxygen and water.

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For *A. fumigatus* only 3-octanone was measured with both methods. The unknown compounds p_642_1/p_683_1 and p_705_3 were only measured with MCC-IMS. In GC/MS measurements, the substances cyclohexanone and isoamyl alcohol occurred in higher concentrations for *A. fumigatus* compared with *C. albicans* and blood agar, but were not detected by MCC-IMS.

Phenethyl alcohol is a known metabolite for *C. albicans* and was described by several other authors.[18,19,36,37] Phenethyl alcohol as morphogenic compound is produced *in situ* by *C. albicans* and *Candida dubliniensis* during planktonic and biofilm growth. Morphogenesis control by chemical signalling molecules is beginning to be highlighted in *Candida* biology. Moreover, phenethyl alcohol has been described as a signalling molecule produced by *C. albicans*, which accelerates and blocks the morphological transition from yeasts to hyphae, respectively.[36]

3-Octanone[38] and isoamyl alcohol are common volatile metabolites of microbes.[39, 40] Isoamyl alcohol is the fusel alcohol derived from leucine and is reported as a *C. albicans* metabolite.[18,36] Other substances described in literature like farnesol[41] or tyrosol[42] produced by *C. albicans* or farnescene by *A. fumigatus*[39] were in the applied experimental setup neither detected with GC/MS nor with MCC-IMS.

All strains investigated in this study were grown on a universal medium (Columbia sheep blood agar). However, different metabolic profiles while using other types of media and growth conditions like temperature and availability of oxygen can be expected.[43–46] In the applied setup, we choose one medium for all strains and found a high reliability of the results. The influence of different media was not subject of the present investigation, but must be considered in the future.

All strains were measured in a growth state with microbial mats at measurement. Other authors have demonstrated that the metabolism depends on the microbiological growth phase.[43] This becomes more relevant, if limits of detection with quantitative cultures are investigated, as the growth phase may be different in lower microbial concentrations. However, the intent was to prove, whether a differentiation in a simple approach, applicable to primary drawn material and direct incubation on a standard medium, is possible. Therefore, we made no efforts on cell quantification, but this will be the subject of future investigations.

We made no attempts to optimise the setup as par example variation of sample gas flow. Volatile organic compounds were determined with MCC-IMS in a sample volume of only 10 ml. We used a sample gas flow of 100 ml min^{-1} to rinse the headspace of the measurement chamber in the sample loop. A reduction of sample gas flow will lead to a higher concentration of volatile compounds and therefore differences might occur in lower microbial count. Further investigations are needed to optimise the MCC-IMS setting and in this context, detection limits have to be defined.

Conclusion

The study describes a basic approach for metabolic profiling of different microbial strains with technology novel approach using MCC-IMS for analysis of the headspace of microbial cultures. It could be demonstrated that an identification of strains on genus level by their volatile metabolic profile is possible. However, a further differentiation on species level for *Candida* species was not possible. There are further investigations necessary to develop a microbiological reference database for volatile metabolic profiles. In exemplary experiments with wild type *C. albicans* and MCC-IMS, the metabolic profile were identically with the VOC pattern of the ATCC 5703 strain used in this study. MCC-IMS is an applicable system for metabolom analysis even in complex volatile mixtures. Additional GC/MS analyses were carried out to identify unknowns in the MCC-IMS data and indicated additional relevant metabolites not detected by MCC-IMS in the present experimental setup.

The MCC-IMS system with the feasibility of rapid analysis of complex gas mixtures without preconcentration was applied in a proof of principle experiment. A discrimination of different strains on genus level by metabolic profile and therefore detection of VOC is feasible for the investigated germs. A differentiation on species level was not possible for the investigated four *Candida* species. The detection of microbial VOCs in breath of patients suffering infections might be a most promising approach to achieve a more early diagnosis of infection and might lead to an early therapy.

Outlook

Obviously, the investigations have to be extended to all considerable microbes to establish a database for their characteristic metabolic profiles. Furthermore, the influence of the media and of the growth phase has to be considered.

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