International Journal of Mass Spectrometry 280 (2009) 162–168

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Biomarkers of *Aspergillus* spores: Strain typing and protein identification

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ARTICLE INFO

Article history:
Received 17 June 2008
Received in revised form 11 August 2008
Accepted 12 August 2008
Available online 28 August 2008

Keywords:
Aspergillus
Spore
MALDI-TOF
Protein
Profiling

ABSTRACT

We applied both matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometric and 1D sodium dodecylsulfate polyacrylamide gel electrophoretic (1D-PAGE) approaches for direct analysis of intact fungal spores of twenty four *Aspergillus* species. In parallel, we optimized various protocols for protein extraction from *Aspergillus* spores using acidic conditions, step organic gradient and variable sonication treatment. The MALDI-TOF mass spectra obtained from optimally prepared samples provided a reproducible fingerprint demonstrating the capability of the MALDI-TOF approach to type and characterize different fungal strains within the *Aspergillus* genus. Mass spectra of intact fungal spores provided signals mostly below 20 kDa. The minimum material amount represented 0.3 μg (10,000 spores). Proteins with higher molecular weight were detected by 1D-PAGE. Eleven proteins were identified from three selected strains in the range 5–25 kDa by the proteomic approach. Hemolysin and hydrophobin have the highest relevance in host–pathogen interactions.

1. Introduction

Mass spectral characterization of bacteria is a well established experimental method and provides many interesting possibilities for the study of various environmental problems, e.g. food and beverages technologies, biodegradation and bacteria typing. In the latter case mass spectrometry uses primarily two approaches, MALDI and ESI (Electrospray Ionization) ionization of the sample, that allow us to study whole cells as well as bacterial proteins, DNA, lipids, exotoxins, lipooligosaccharides and peptidoglycans [1,2]. The typing of various intact microorganisms and the use of different mass spectral protocols have been extensively reviewed [3,4], mostly focused on the analysis of bacteria.

On the other hand, mass spectral characterization of intact fungal spores is not yet well described in the literature. Fungal spores are typically larger than bacterial cells and have rigid walls which structurally, but not chemically, resemble plant cells [5]. Different types of spores are formed following either asexual (the products of mitosis) or sexual (the products of meiosis) processes. Some fungal spores are able to survive at extreme conditions (elevated temperature and/or pressure) and can cause allergies with irritations of the eyes, nose, throat, and lungs [6]. Systemic mycotic infections or intoxications are also caused by molds and become a serious clinical problem for immunocompromised individuals [7]. Invasive infections caused by *Aspergillus*, mainly *Aspergillus fumigatus*, have one of the highest mortality rates, where 90–100% of immunocompromised patients die despite treatment [8]. *Aspergillus* is the second most common fungal pathogen after *Candida*. Aspergillosis and candidiasis together account for more than 80% of all fungal episodes in both bone marrow and solid organ transplantations [9].

This work was inspired by a high-sensitivity identification of *Bacillus* spores by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry [10]. Also, preliminary results in *Penicillium* spore characterization by MALDI-TOF with different matrices demonstrated its ability for fungal spore classification [5]. Moreover, certain toxigenic strains of *A. flavus* and *A. parasiticus*, known to contaminate food and produce toxic secondary metabolites with mutagenic and carcinogenic effects (i.e. aflatoxins), have also been characterized by MALDI-TOF mass spectrometry [11].

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doi:10.1016/j.ijms.2008.08.012

Table 1
Aspergillus strains involved in the study

Name of species	Number	Source	Year of isolation
A. fumigatus	CCF3227	Laboratory air	2000
A. fumigatus	CCF1187	Throat of a patient	1970
A. fumigatus	GR-1	Mouse aspergilloma	2000
A. fumigatus	CZ-2	Mouse aspergilloma	2001
A. fumigatus	CCF3623	Human maxillary cavity	2003
A. fumigatus	CCF1293	Throat of a miner	1968
A. fumigatus	CCF1292	Throat of a miner	1967
A. fumigatus	CCF1059	Root of sugar beet	1965
A. niger	MZ-3	Laboratory air	2002
A. niger	CCF3264	Mouldy twist from wheat flour	1999
A. niger	CCF2477	Patented strain	1967
A. niger	CCF1297	Throat of a miner	1967
Emericela nidulans	CCF3379	Human auditory meatus	2003
Emericela rugulosa	CCF3089	Human pericardium	1998
A. ochraceus	CCF1893	Cave air	1983
A. flavipes	CCF2026	From archives	1986
A. flavus	CCF3201	Black tea	2000
A. flavus	CCF2497	Seed of *Cicer arietinum*	1987
A. flavus	CCF1288	Throat of a miner	1967
A. flavus	CCF1058	Root of sugar beet	1965
A. parasiticus	CCF3137	Soil of tropical rain forest	2000
A. parasiticus	CCF1299	Throat of a miner	1967
A. oryzae	CCF1602	Food	1977
A. oryzae	CCF1066	ATCC10063	1996

Legend: CCF, Culture Collection of Fungi; ATCC, American Type Culture Collection; GR-1, CZ-2 and MZ-3 are fungal isolates from own collection (GR-1, CZ-2 were provided by M. Hajduch, MZ-3 by M. Zabka).

In this paper we report on the analysis of intact *Aspergillus* spores by MALDI-TOF mass spectrometry. We probed spore protein profiles by both the mass spectral and gel approaches. We tuned the experimental parameters and built a mass spectral database with twenty four *Aspergillus* strains. Eleven fungal proteins were identified by tandem mass spectrometry in three selected strains.

2. Experimental

2.1. Aspergillus cultivation and spore harvesting

Dry spores of *Aspergillus* strains (Table 1) were obtained at a Biosafety Level two laboratory. Cultivation was carried out in conical Erlenmeyer flasks at room temperature for 21 days with sterilized barleycorn. The inoculum was prepared from a culture performed on Sabouraud-dextrose agar in Petri-dishes (7 days). Spore collection from the fully sporulated culture in conical Erlenmeyer flasks was carried out by a vacuum collector covered with a 1.0 μm nitrocellulose membrane filter (Whatman, UK) and a stream of nitrogen.

2.2. Optimization of MALDI-TOF-compatible protein extraction protocol

Six variants of spore sample treatment were tested with *A. fumigatus* CCF3227 to increase both the quantitative and qualitative abundance of detected proteins (Table 2A). Two conditions were tested for MALDI-TOF analysis: variable amount of spores on the target mixed with a single MALDI matrix (Table 2A, protocol number 2, CHCA) and variable MALDI matrix with a fixed spore amount (protocol number 1, 0.1 mg of spores on the target). In both these experiments the aqueous spore suspension was dried in SPEED-VAC DNA-110 (ThermoSavant, USA) and the dry pellet was diluted in 2 μL of matrix solution [20 mg/mL of matrix in 50% aqueous acetonitrile/0.1% trifluoroacetic acid (TFA, v/v)]. 1 μL of the mixture was directly subjected on the MALDI target and the droplet was allowed to dry at ambient temperature.

Further, we optimized protein extraction conditions as shown in Table 2A (protocols no. 3–6). An aqueous suspension of intact spores (equivalent to 0.1 mg) was dried in SPEED-VAC, diluted

Table 2
Optimizing the protein extraction conditions (*Aspergillus fumigatus* CCF3227 exclusively used) for MALDI-TOF (A) and 1D-electrophoresis (B) profiling

A	Optimized parameter	Variable value/type of optimized parameter					
1	Type of MALDI-TOF matrix	CHCA	FA	DHB	SA		
2	Amount of spores [μg]	100	10	2.5	0.62		
3	TFA extraction [% (v/v)]	0.1	0.2	0.5	1	1.5	2
4	Aqueous MeCN extraction [% (v/v)]	0	10	20	40	60	80
5	Combination of 40% aqueous MeCN with TFA extraction [% (v/v)]	0.1	0.2	0.5	1	1.5	2
6	Sonication strength	3 × 2 s (50 W, amplitude 60) by a probe				15 min in a water bath	

B	Extraction solution	Sonication conditions
1	T50E1U8D100S1	Probe 3 × 2 s (50 W, amplitude 60), 1 min break on ice
2	T50E1U8D100S1	15 min in a water bath
3	40% MeCN (v/v)	15 min in bath
4	40% MeCN/0.1% TFA (v/v)	15 min in bath

Legend A: CHCA, α-cyano-4-hydroxy-cinnamic acid; DHB, dihydroxybenzoic acid; FA, ferulic acid; SA, sinapinic acid; TFA, trifluoroacetic acid; MeCN, acetonitrile.
Legend B: Composition of T50E1U8D100S1 buffer: 50 mM Tris-Cl, 1 mM ethylene diamine tetra-acetic acid, 8 M urea, 100 mM dithiothreitol, 1% sodium dodecylsulfate, pH 8.0; MeCN, acetonitrile; TFA, trifluoroacetic acid.

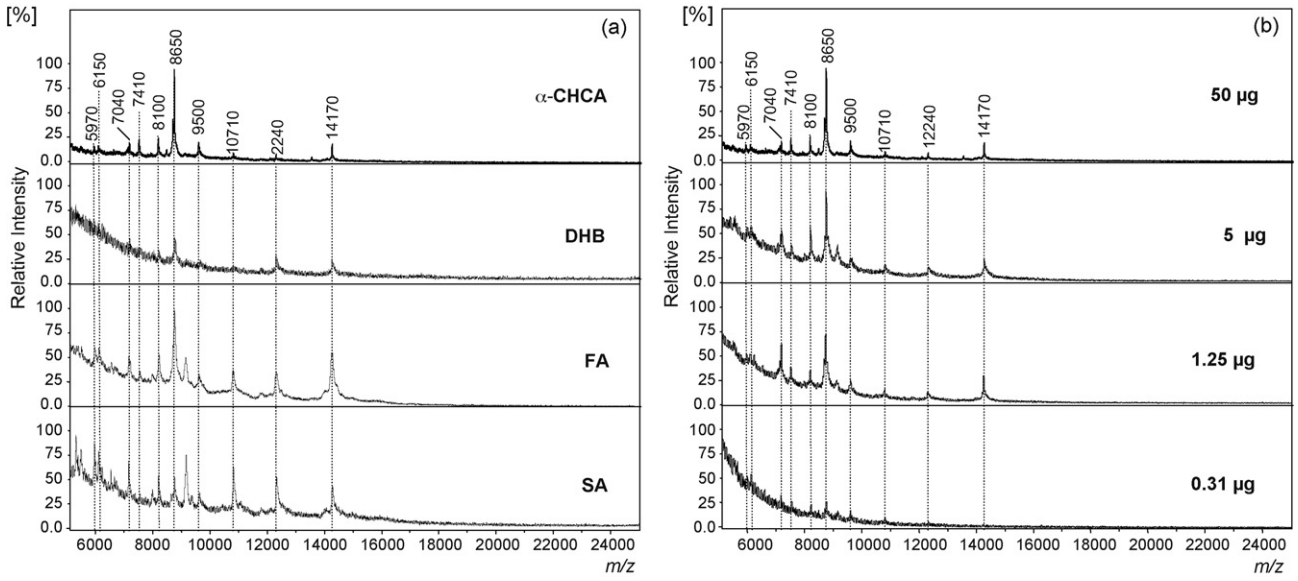

Fig. 1. MALDI-TOF mass spectra of *Aspergillus fumigatus* CCF3227: (a) 50 µg of spores in four matrices: α-cyano-4-hydroxy-cinnamic acid (CHCA), dihydroxybenzoic acid (DHB), ferulic acid (FA), or sinapinic acid (SA), respectively. (b) Four different amounts of spores in CHCA matrix: 50, 5, 1.25, or 0.31 µg, respectively.

in selected extraction solution, sonicated for 15 min in a water bath sonicator (Table 2A, protocols 3 and 4) and after centrifugation (MiniSpin Eppendorf, 13,000 RPM, 5 min) the supernatant was dried in SPEED-VAC. The dry residue was processed with the CHCA matrix as described previously and analyzed by MALDI-TOF mass spectrometry. In protocol 6 (Table 2A) we tested the influence of sonication power on the extent both of protein extraction as well as spore destruction. The suspension of intact spores (equivalent to 0.1 mg) was sonicated either for 15 min in a water bath sonicator or three times for 2 s by sonication probe (50 W, amplitude 60, Cole-Parmer, USA) interlaced with one minute of cooling on ice. Then the suspension was let to dry in SPEED-VAC and processed for MALDI-TOF analysis as described previously.

Mass spectra were measured in a linear mode on a MALDI-TOF mass spectrometer BIFLEX (Bruker-Franzen, Bremen, Germany) equipped with a SCOUT 26 sample inlet, a nitrogen laser (337 nm) (Laser Science, Cambridge, USA) and a gridless delayed extraction ion source. Spectra were accumulated from 100 to 150 laser shots. Positive-ion mass spectra were calibrated externally using the average masses of $[M+2H]^{2+}$, $[M+H]^{+}$, and $[2M+H]^{+}$ lysozyme ions. Mass accuracy better than 0.2% was usually achieved.

2.3. Protein extraction optimization for 1D-PAGE

To obtain a comparative protein profile to the mass spectral approach we also used 1D-electrophoresis (Table 2B). An aqueous solution of intact spores (*A. fumigatus* CCF3227, 0.5 mg) was

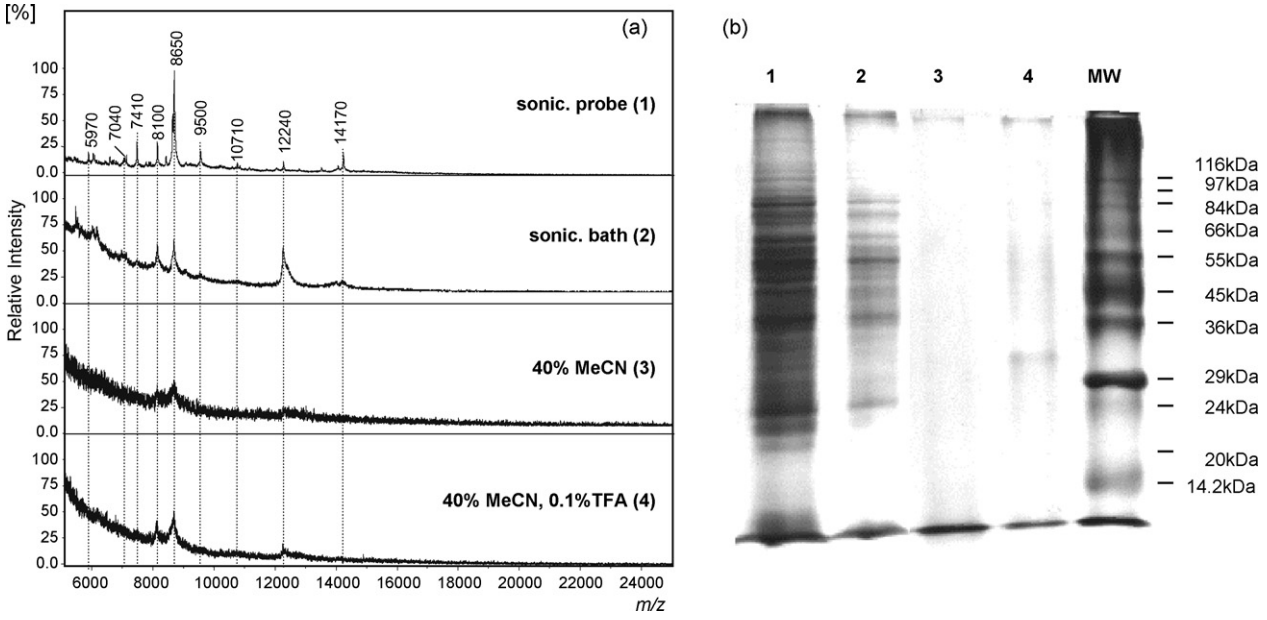

Fig. 2. MALDI-TOF mass spectra (a) or 1D-electrophoresis (12% gel, silver staining, MW—Sigma wide-range molecular weight standards) (b) of *Aspergillus fumigatus* CCF3227 spores under various protein extraction conditions (Table 2).

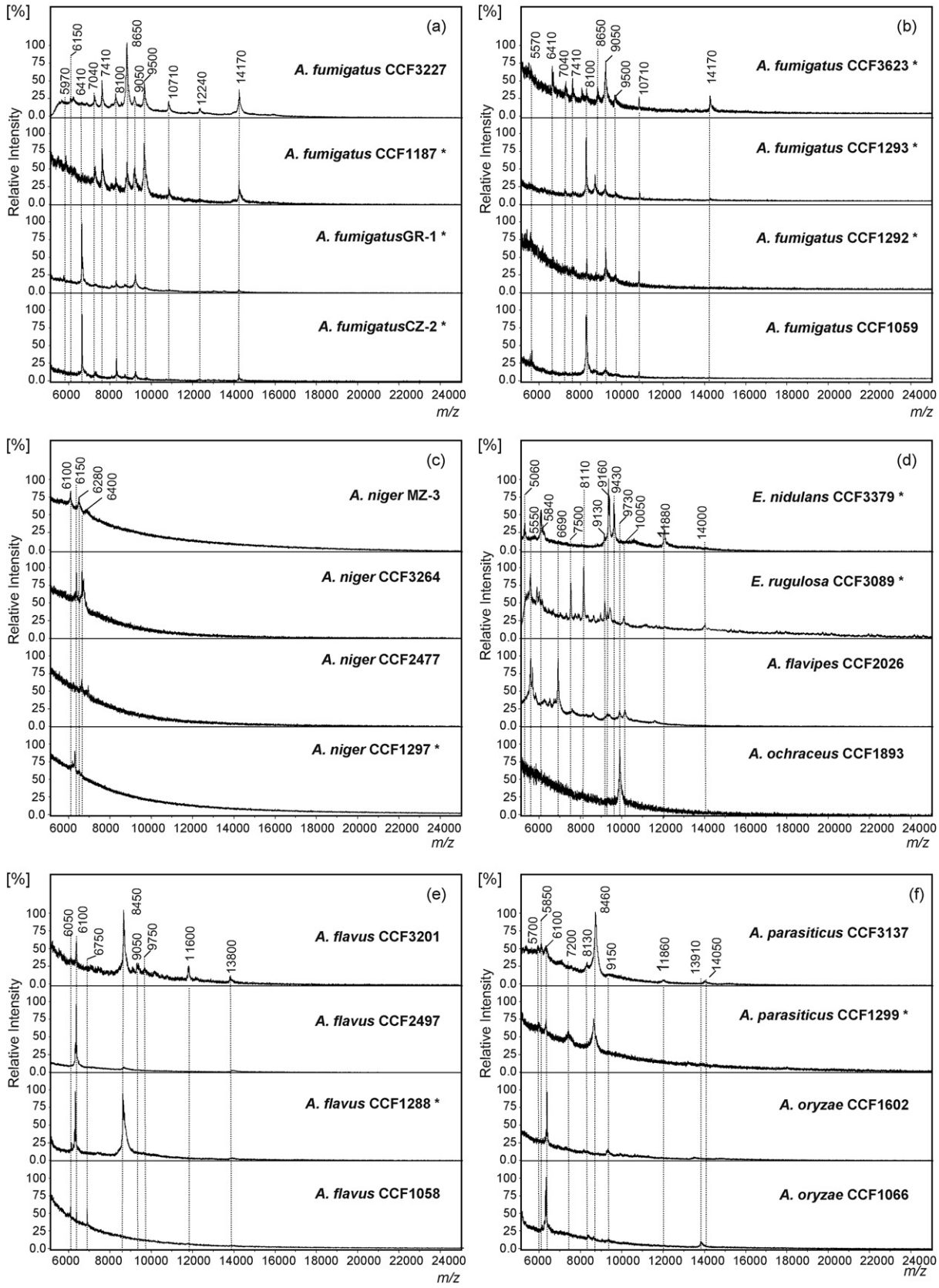

Fig. 3. MALDI-TOF mass spectra of *Aspergillus* spores under study.

Table 3
MALDI-TOF mass spectral characterization of *Aspergillus fumigatus* strains

Species	Accession number	*m/z* values									
		6410	7040	7410	**8100**	8650	**9050**	9500	**10710**	12240	14170
A. fumigatus	CCF3227	–	±	+	**+**	++	±	+	±	±	+
	CCF1187	–	±	++	±	+	**+**	++	**+**	–	+
	GR-1	++	–	–	±	–	**+**	±	±	–	±
	CZ-2	++	–	–	**+**	–	**+**	±	±	±	±
	CCF3623	+	±	+	±	±	**++**	±	**+**	–	+
	CCF1293	–	±	±	**++**	+	**+**	±	±	–	±
	CCF1292	–	–	±	**+**	–	**+**	±	**+**	–	–
	CCF1059	–	–	–	**++**	–	±	–	±	–	–

Legend: (–) not observed, (±) weak (5–20%), (+) medium (20–50%), (++) abundant (50–100%) relative intensity; signals in bold represent species common to all strains.

dried in SPEED-VAC. Dry residue was then diluted in selected extraction solution, sonicated either for 15 min in a water bath sonicator or three times for two seconds (50 W, amplitude 60; Cole-Parmer, USA) with a sonication probe, interlaced with one minute of cooling on ice. After centrifugation (MiniSpin Eppendorf, 13,000 RPM, 5 min) the supernatant was directly added to a sample buffer [50 mM Tris-Cl pH 6.8, 100 mM DTT, 2% SDS (w/v), 0.1% bromophenol blue (w/v), 10% glycerol (v/v)] and loaded on 12% or 15% polyacrylamide gel in the presence of sodium dodecylsulfate. After protein separation on 1D-gel the protein bands were detected by silver [12] or Coomassie Brilliant Blue R-250 [(CBB, 0.25% (w/v)] staining.

2.4. Protein identification by enzymatic in-gel digestion and μLC–MS/MS analysis

CBB-stained bands were excised from the gel, cut into small pieces and destained by 0.1 M 4-ethylmorpholine acetate (pH 8.1) in 50% acetonitrile (v/v, MeCN). The gel pieces were further washed with water, shrunk by dehydration in MeCN and reswelled again in water. After the supernatant removal the gel was partly dried in a SPEED-VAC concentrator and then reconstituted in a cleavage buffer containing 0.05 M 4-ethylmorpholine acetate, 10% MeCN (v/v), and sequencing grade trypsin (20 ng/mL; Promega). After overnight digestion at 37 °C, the resulting peptides were extracted twice to 40% MeCN/0.1% trifluoroacetic acid (v/v). The extracts were pooled, completely dried, dissolved in 5% ACN/0.5% acetic acid (v/v) and loaded onto a column (0.180 mm × 100 mm) packed with MAGIC C18 (5 μm, 200 Å) RP resin (Michrom BioResources) and separated using a gradient from 5% MeCN/0.5% acetic acid to 40% MeCN/0.5% acetic acid for 70 min. The column was connected directly to an LCQ^{DECA} ion trap mass spectrometer (ThermoFisher, San Jose, CA) equipped with a nanoelectrospray ion source. Full scan spectra were recorded over the mass range 380–1600 Da followed by MS/MS scans of the three most intense ions in the preceding full scan. Dynamic exclusion was set with two repeat counts, repeat duration of 30 s and 3 min exclusion duration window. Peak lists in DTA format were created by lcq_dta.exe program (ThermoFisher, San Jose, CA) with the following criteria: MW range 700–3300 Da; minimum number of ions 20; minimum TIC 5×10^4; precursor charge state +1, +2, +3; and grouped scans 1. Peak lists were searched against a UniProtKB protein database subset of the Fungi/*Metazoa* group using SEQUEST^{TM} software (ThermoFisher, San Jose, CA) with the following settings: enzyme chemistry-trypsin, missed cleavages 2, no modification of cysteine, variable single oxidation of methionine, precursor-ion tolerance 2 Da, fragment-ion tolerance 0.8 Da. All MS/MS spectra assignments were validated manually.

3. Results and discussion

3.1. Tuning the extraction and MALDI acquisition conditions

In our study, four MALDI matrices were probed with *A. fumigatus* CCF3227 spores (Fig. 1). In this comparison the spores (50 μg) were extracted to 50% acetonitrile (v/v). Excluding the DHB, all used matrices provided rich ion profiles. The best signal/noise ratio and the narrowest peaks with little signal suppression were obtained in the CHCA matrix, which was then selected for all subsequent experiments. The same matrix was used for bacterial cells and their spores [13]. In the contrast, other authors also used the DHB, [2-(4-hydroxyphenylazo)]benzoic acid and 3,5-dimethoxy-4-hydroxycinnamic acid matrices for typing the *Penicillium* spp., *Scytalidium dimidiatum* and *Trychophyton rubrum* spores in the 2–13 kDa mass range [5].

To determine the limit of spore detection in MALDI-TOF analysis, variable quantities of *A. fumigatus* CCF3227 spores were used

Table 4
MALDI-TOF mass spectral characterization of *Aspergillus flavi* group strains (*A. flavus*, *A. oryzae* and *A. parasiticus*)

Species	Accession number	*m/z* values							
		5850	6050	**6100**	6750	8460	11600	13800	14050
A. flavus	CCF3201	–	±	+	–	++	±	±	–
	CCF2497	–	–	++	–	±	–	±	–
	CCF1288	–	±	++	–	++	–	±	–
	CCF1058	–	±	±	+	–	–	–	–
A. oryzae	CCF3137	±	–	±	–	++	±	–	±
	CCF1299	±	–	±	–	+	–	–	–
A. parasiticus	CCF1602	–	–	++	–	–	–	–	–
	CCF1066	–	–	++	–	±	±	+	–

Legend: (–) not observed, (±) weak (5–20%), (+) medium (20–50%), (++) abundant (50–100%) relative intensity; signals in bold represent species common to all strains.

(a)

Band	NCBI acc.n.	Protein name [species]
Af1-3	gi|19577409	hydrophobin [A. fumigatus]
Af4	gi|19743612	nucleoside diphosphate kinase [E. nidulans]
	gi|1220098	hemolysin [A. fumigatus]
Af5	gi|5326835	superoxide dismutase [Cu-Zn] [A. fumigatus]
An2	gi|2632155	histone H2A[A. niger]
An3	gi|19743612	nucleoside diphosphate kinase [E. nidulans]
Er2	gi|2632155	histone H2A[A. niger]
	gi|19743612	nucleoside diphosphate kinase [E. nidulans]
Er3	gi|19743612	nucleoside diphosphate kinase [E. nidulans]
Er4	gi|40743823	histone H2B[A. nidulans]
	gi|4322946	cyclophilin-like peptidyl prolyl cis-trans isomerase [A. niger]

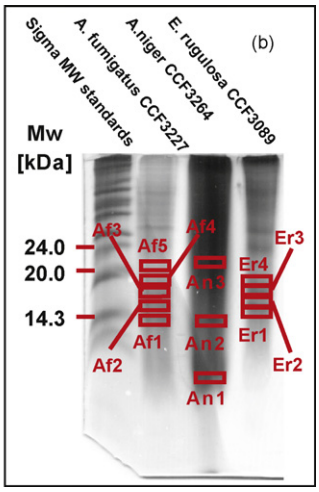

Fig. 4. The list of identified low MW proteins (a) of 1D-PAGE (15% gel, CBB R-250 staining, MW—Sigma wide-range molecular weight standards) separated protein bands of three selected *Aspergillus* strains (b).

(0.15–100 μg). Fig. 1b demonstrates decent ion signals even at spore amounts as low as 0.31 μg (dry material) corresponding to signal/noise ratio >2.5 for *m*/*z* 8650. This value corresponds to about 6500 fungal spores as determined in a Neubauer counting chamber and is consistent with the sensitivity reported for *Bacillus* species [10]. Interestingly, some authors claim even 1000 times higher sensitivity [11]. In our study some tested strains provided only poor signals. Hence, 50 μg of spore material was used in typing experiments (see below).

Further, we tested several protein extraction protocols such as the step gradient of aqueous acetonitrile with or without trifluoroacetic acid and sonication treatment (Fig. 2). *A. fumigatus* CCF3227 spores (0.1 mg) were used in this assay. Optimal "extraction" conditions include ultrasonic treatment with a probe running at high power (see Section 2). Cells had to be cooled down during the treatment to minimize biomolecular thermal degradation. The addition of 0.1% TFA (v/v) helps both MALDI-TOF and 1D-PAGE analyses. Mutual comparison of MALDI-TOF and 1D-PAGE protein typing results defines the following benefits and drawbacks of both approaches. MALDI-TOF analysis in concert with the reported extraction protocol discriminates species with molecular weight higher than 15,000 Da. However, MALDI is much more sensitive (about 1600 times in this particular case). On the other hand, the less sensitive 1D-PAGE analysis of *Aspergillus* strains enables one to use some proteomic approach for subsequent fungal protein identification (see below).

Finally, we also evaluated the influence of spore aging/storage on the protein profile. The study was carried out with *A. fumigatus* CCF3227. We evaluated MALDI-TOF profiles of spores stored as aqueous suspension at −20 °C for variable times (15 months, 30 months, or 5 years) of storage or we analyzed freshly harvested spores. Interestingly, we did not see any significant qualitative changes in mass spectral profiles (data not shown). This result indicates that fungal spore material can be used for typing purposes, as the biomolecular organic content remains almost constant.

3.2. Fungal strain typing

Optimal sample preparation conditions were used for the acquisition of reference spectra of 24 *Aspergillus* strains. In the optimized protocol the intact spores were processed as aqueous suspension (0.1 mg, dry material), destroyed by sonication probe, dried, mixed with the CHCA matrix solution and directly subjected on the MALDI target. Shared features in MALDI-TOF mass spectra (Fig. 3) indicate the phylogenetic and morphologic proximity of individual *Aspergillus* genera.

A. fumigatus set of strains is well characterized by common biomarker signals at *m*/*z* 8100, 9050 (Table 3) and 10,710, that are often accompanied by ions at *m*/*z* 9500 and 14,170 (Fig. 3a and b). On the contrary, *Aspergillus niger* strains exhibit a characteristic peak pattern in the range *m*/*z* 6100–6400 as illustrated in Fig. 3c. The poor signal of *A. niger* mass spectra might be rationalized by the presence of black pigments detected in low molecular weight MALDI-TOF mass spectra on the spores (data not shown), possibly interfering with the ionization process of biomolecules.

It is worth mentioning that MALDI-TOF spectra of other fungal strains could also be phylogenetically related as examined by *A. flavi* group strains shown in Table 4. The analysis of four *A. flavus* strains (Fig. 3e) reveals two shared markers at *m*/*z* 6100 and 8450. Whereas the species at *m*/*z* 6100 can also be observed both in *A. parasiticus* and *A. oryzae* strains (Fig. 3f), the latter ion *m*/*z* 8450 was present in the mass spectra just of *A. parasiticus* and represents the base peak there. The observation of a diagnostic marker at *m*/*z* 6100 was not a surprise: it has already been reported in the literature for multiple *Aspergilli* [11]. The *A. flavus* and *A. oryzae* spectra have clearly provided this peak, in contrast to suppression of this signal in other *Aspergillus* strains. On the other hand, *A. parasiticus* strains have revealed a signal at *m*/*z* 5850 which was not present in *A. flavus* and *A. oryzae* strains and could be used for distinguishing purposes. Interestingly, this relationship is in agreement with the origin of *A. oryzae* proposed from *A. flavus* [14] and *A. parasiticus* from *A. soyae* [11].

Although almost all described species are characterized by quite a high degree of morphological similarity, the fungal species differ in the production of carcinogenic aflatoxins [15]. While about 30–85% of *A. flavus* and *A. parasiticus* strains maintained in the American Type Culture Collection (ATCC) had produced detectable amounts of aflatoxins, no strain of *A. oryzae* is reported as an aflatoxin producer [16]. These data were supported also by DNA Southern blot analysis [17]. Coincidentally, MALDI-TOF mass spectra of *A. flavus* and *A. parasiticus* have shown the following interesting similarity. Two *A. flavus* (CCF1288, CCF3201) and both *A. parasiticus* (CCF1299, CCF3137) strains revealed an ion at *m*/*z* 8450. We cannot exclude that the generation of this ion is consistent with the production of aflatoxins reported by [18].

Other *A. flavus* (CCF1058, CCF2497) strains do not generate this biomarker.

All typing experiments were performed in triplicate and a constant pattern was usually recorded.

3.3. Fungal protein identification

There are several proteomic approaches to identify proteins that correspond to signals detected in MALDI-TOF spectra. In case of an organism with completely sequenced and reported genome, the molecular weights of a microorganism's proteins, obtained from its mass spectrum, are compared to those retrieved from the corresponding genome and/or protein databases [19]. Because our selected *Aspergilli* strains (*A. fumigatus*, *Emericella rugulosa* and *A. niger*) did not belong to organisms with a completely sequenced genome, a bottom-up proteomic approach was used as a platform for fungal protein identification in the range of molecular weight 5–25 kDa (Fig. 4). In-gel trypsin digestion was performed on selected 1D-PAGE strips prior to LC–MS/MS analysis. Proteins were identified by SEQUEST™ algorithm and the results are summarized in Fig. 4a. In *A. fumigatus* we detected four bands of hydrophobin, indicating multiple forms with different posttranslational modifications (Table S1, Supplementary Information Section). Like hemolysin, hydrophobin is an accepted fungal virulence factor. Another enzyme found in *A. fumigatus* was superoxide dismutase [Cu–Zn]. In *Emericella* as well as in *A. niger* we identified different forms of histones (H2A and H2B family). Nucleoside diphosphate kinase was ubiquitous in all three examined strains. Protein identification is demonstrated in the case of hydrophobin tryptic peptide as an example (Fig. S1, Supplementary Information Section), $[M+2H]^{2+}$ at m/z 566.3.

4. Conclusion

The MALDI-TOF protein profiling of intact fungal spores represents a fast approach for microorganism identification. Mass spectrometry can generate a unique fingerprint, which can be used for strain classification. This approach was successfully used for distinguishing *A. flavus*, *A. parasiticus*, *A. oryzae* and *A. fumigatus* strains. Selected protein biomarkers could speculatively correlate with the production of some secondary metabolites, e.g. aflatoxins [18]. Similarly, protein biomarkers, as well as some small secondary metabolites, might be correlated with phylogenetic evolution and used in metabonomic applications.

Although no general and specific marker in all *Aspergillus* isolates with a clinical origin (Fig. 4) was found in this work, the set of identified protein molecules present on fungal spores can potentially be used in human/veterinary medical mycology for early diagnosing purposes.

Acknowledgments

This paper is dedicated to our friend and colleague, Prof. Zdenek Herman. This work was supported by the Czech Science Foundation (203/05/P575), the Ministry of Education, Youth, and Sports of the Czech Republic (LC07017, MSM 6198959216) and an institutional research concept of the Institute of Microbiology, Academy of Sciences of the Czech Republic (AV0Z50200510). We also thank the Culture Collection of Fungi (CCF), Charles University, Prague, Czech Republic, for providing the *Aspergillus* strains. The authors would like to thank Douglas E. Markham for his helpful remarks and proofreading.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2008.08.012.

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