DETECTION OF SPECIFIC SPECTRAL MARKERS OF *COXIELLA BURNETII* ISOLATES BY MALDI-TOF MASS SPECTROMETRY

Ľ. ŠKULTÉTY[1], L. HERNYCHOVÁ[2], E. BEREGHÁZYOVÁ[1], K. SLABÁ[1], R. TOMAN[1*]

[1]Laboratory for Diagnosis and Prevention of Rickettsial and Chlamydial Infections, Institute of Virology, Slovak Academy of Sciences, 845 05 Bratislava, Slovak Republic, [2]Institute of Molecular Pathology, Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czech Republic

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Summary. – Specific markers for *Coxiella burnetii* (*C.b.*) isolates RSA 493, Priscilla, and BUD were detected using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS). The method revealed noticeable differences in the ion signal profiles of the isolates in the mass range of 3–18 kDa. The number of characteristic ions for RSA 493, BUD, and Priscilla was 24, 15, and 7, respectively. The specific markers were compared against *C.b.* database using the Tag-Ident proteomics tool. For the isolates RSA 493, Priscilla and BUD there were identified 11, 5 and 3 potential biomarkers, respectively. This method represents a powerful tool for the rapid, sensitive, and differential characterization of *C.b.* isolates and is a good candidate for phyloproteomic approaches.

Key words: biomarkers; Coxiella burnetii; isolates; MALDI-TOF mass spectrometry; spectral markers

Introduction

Rapid and reliable detection and identification of microorganisms is of great importance in numerous fields like clinical medicine, public health, food production, and biotechnology. Recently, a number of studies have shown a potential of MALDI-TOF MS in this area (Smith *et al*., 2001; Jackman and Moss, 2004; Keys *et al.,* 2004; Seto *et al*., 2005). This method is based on the determination of unique spectral markers as indicators of the genus, species, or even strain of the microorganism. Up to now, this approach has not received a widespread application in the detection of obligate intracellular bacteria.

C.b. is an extremely infectious, obligate intracellular, and highly pleomorphic bacterium causing Q fever, a zoonotic disease, which is transmissible from animals to humans (Baca and Paretsky, 1983). The most common acute form of Q fever is manifested in man as a flue-like illness, atypical pneumonia and less frequently as a granulomatous hepatitis with a significant incidence of neurologic complications. A persistent *C.b.* infection leads to a chronic form of the disease with endocarditis, liver complications, and possibly fatigue, which may not be manifested until much later (Maurin and Raoult, 1999). In animals, Q fever affects livestock causing pneumonia and reproductive disorders as abortion, stillbirth, placentitis, endometritis, and infertility (Bildfell *et al.,* 2000; Moeller, 2001; Arricau-Bouvery and Rodolakis, 2005). An unambiguous clinical diagnosis of the disease is quite difficult, because several clinical symptoms of Q fever in humans are similar to commonly occurring infections. Many serological methods are used for the rapid and sensitive diagnosis of the disease, but ambiguous results are frequently obtained (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005; Slabá *et al.,* 2005).

We report here the ion profiles of three *C.b.* isolates, RSA 493, Priscilla and BUD using MALDI-TOF MS. This is the first step toward developing a novel approach to the detection of *C.b.* and its strains/isolates by MS technique. Using a database search, a tentative identification of specific spectral markers for these isolates was accomplished.

[*]Corresponding author. E-mail: virutoma@savba.sk; fax: +4212-54774284.

Abbreviations: *C.b.* = *Coxiella burnetii*; MALDI-TOF = matrix assisted laser desorption/ionization time of flight; MS = mass spectrometry; m/z = mass-to-charge ratio

Materials and Methods

C.b. isolates. RSA 493 (isolated from a *Dermacentor andersoni* tick in Montana, USA, 1937), Priscilla (isolated from a placenta of aborting goat in Montana, USA, 1980), and BUD (isolated from the blood of a patient with acute Q fever in Budulov, Slovakia, 1969) were obtained from the WHO Collaborating Centre for Rickettsial Reference and Research, Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovak Republic. The isolates underwent three passages in embryonated hen eggs and were in phase I.

Purification of C.b. isolates. The three isolates were propagated in embryonated hen eggs, inactivated with phenol and purified by differential centrifugation and ether as described (Škultéty *et al*., 1998).

MALDI-TOF MS. The cellular material of the isolates (5 mg) was resuspended in 1 ml of 70% acetonitrile-water containing 0.5% trifluoroacetic acid. After vigorous extraction by vortexing (5 mins), the mixture was centrifuged (12,000 x g, 15 mins, 10°C) and the cell free supernatant was concentrated in Speed Vac (Eppendorf), until the volume was reduced to 10 μl. The concentrated extract (1 μl) was deposited on a hydrophobic sample slide and a matrix solution containing sinapinic acid (10 mg/ml) in aqueous 30% acetonitrile with 0.5% trifluoroacetic acid (1 μl) was added, air-dried, and introduced into a Voyager-DE-STR mass spectrometer (Perseptive Biosystem, USA) equipped with a delayed extraction. The spectra were recorded in a positive ion mode in a linear configuration at an acceleration voltage of 25 kV, 93% grid voltage, 0.15% guide wire, 320 ns delay time, and a low mass gate at 1000 m/z. Internal and external mass markers were used to ensure mass accuracy. Each mass spectrum was obtained by averaging 200 laser shots. The Data Explorer Program (Matrix Science) was used to view and process the data. An automated spectra processing with the aim to extract peaks and analyze/compare spectral markers was carried out using a small in-house written program**.** The spectral markers were considered identical in the mass range of 35 Da. The identification of prominent ions was done using the Tag-Ident proteomics tool (http://www.expasy.org/tools/tagident.html). The marker mass values were searched against UniProtKB/Swiss-Prot and UniProtKB/TrEMBL databases (Swiss Institute of Bioinformatics) at an allowed 2% mass difference and full pI range.

Table 1. Characteristic ions of *Coxiella burnetii* isolates in the range of 3–18 kDa obtained by MALDI-TOF MS

RSA 493		Priscilla		BUD	
m/z	Intensity (%)	m/z	Intensity (%)	m/z	Intensity (%)
–	–	3016	15	–	–
–	–	3259	15	3264	17
3728	15	–	–	–	–
3912	26	–	–	–	–
4501	100	–	–	–	–
4613	36	–	–	–	–
4675	44	–	–	–	–
–	–	–	–	5109	23
5154	15	–	–	–	–
5282	32	5311	15	–	–
–	–	–	–	5471	17
–	–	5513	55	–	–
–	–	5620	21	–	–
5811	30	–	–	–	–
6132	15	–	–	–	–
6553	31	–	–	–	–
6894	98	–	–	6895	75
7961	30	–	–	–	–
8190	23	–	–	8179	25
10009	46	–	–	10006	30
10226	41	–	–	10231	35
10503	30	–	–	10500	41
–	–	10561	100	–	–
10941	44	–	–	10959	39
–	–	11010	17	–	–
11159	77	–	–	11145	100
–	–	–	–	11442	16
11479	21	–	–	11480	26
12265	50	–	–	12263	22
13399	22	–	–	–	–
15273	30	–	–	15267	24
16388	54	–	–	16378	40

m/z = mass to charge ratio. Masses represent $[M + H]^+$ ions.

Results and Discussion

MALDI-TOF MS of the *C.b.* isolates RSA 493, Priscilla, and BUD revealed characteristic differences in their ion signal profiles at the mass range of 3–18 kDa (Table 1). Initially, it was evident that Priscilla had a special position among the investigated isolates since only 7 ions were detected under the given experimental conditions. For RSA 493 it was found the highest number of ions (24) and for BUD it was recorded a lower number (15). For Priscilla, all ions except that at m/z 3259 had a very high diagnostic value, as they were located in the m/z ranges far away from other ions generated by RSA 493 and BUD. In addition, two of them at m/z 10561 and 5513 had a high intensity**.** If we apply similar criteria to RSA 493 and BUD isolates, it is evident that RSA 493 was superior to BUD, because it gave a whole series of unique ions of a relatively high intensity in the m/z range 3700–6600. In the higher m/z range, only two spectral markers at m/z 7961 and 13399 were obtained. For BUD, lower intensity ions at m/z 5109, 5471, and 11442 appeared to be quite distant from the mass range of other isolates. The high intensity ions at m/z 10959, 11145, and 16378 were also unique. However, the observed mass difference compared to the ions exhibited by RSA 493 was too low for considering them as the specific spectral markers.

The Tag-Ident proteomics tool was applied to identify the biomarkers of isolates. After filtering the *C.b.* ORF

Table 2. Potential biomarkers of *Coxiella burnetii* isolates revealed by Tag-Ident proteomics

Isolate	Observed mass	Theoretical mass	Acc. No.[a]	Locus	Protein
RSA 493	3728	3723	Q83B76	CBU 1637	Hypothetical
	3912	3915	Q83EC4	CBU 0401	Hypothetical
	4501	4507	Q83ED1	CBU 0394	Hypothetical
	4613	4636	Q83BC0	CBU 1592	Hypothetical
	4675	4670	Q83BV9	CBU 1378	Hypothetical
	5154	5157	Q83EC2	CBU 0403	Hypothetical
	5811	5809	Q83F70	CBU 0078	Hypothetical
	6132	6132	Q83CZ0	CBU 0961	Hypothetical
	6553	6566	Q83B27	CBU 1692	Conserved domain protein
	7961	7965	Q83CQ8	CBU 1050	Carbon storage regulator homolog 2
	13399	13402	Q83DQ2	CBU 0644	Conserved domain protein
Priscilla	3016	2953	Q06HF8	-	Hypothetical
	5513	5499	Q83F04	CBU 0149	Hypothetical
	5620	5632	Q83E89	CBU 0438	Hypothetical
	10561	10566	Q83EY9	CBU 0168	Putative acyl carrier protein
	11010	11016	Q83DI6	CBU 0745	Ribosomal subunit interface protein
BUD	5109	5111	Q83AB5	CBU 1989	Hypothetical
	5471	5477	Q06HD1	-	Hypothetical
	11442	11369	Q83A26	CBU 2085	Conserved domain protein

[a]Accession number in the UniProtKB/Swiss-Prot and UniProtKB/TrEMBL databases.

products, an optimum protein list was created. Eleven potential biomarkers have been identified for RSA 493, five for Priscilla, and three for BUD (Table 2). However, most of them are hypothetical proteins of unknown function or homology. Only three conserved domain proteins (CBU 0644, CBU 1692, and CBU 2085) and three ORF products with fair characterization (CBU 1050, CBU 0168, and CBU 0745) were found. It should be mentioned that only *C.b.* RSA 493 was fully sequenced (Seshadri *et al*., 2003). Therefore, the database search of mass values obtained for Priscilla and BUD might lead to some ambiguous protein identifications at present.

In conclusion, we showed that the MALDI-TOF MS ion profiles exhibited by three *C.b.* isolates of different origin differed considerably from each other. Thus, the MALDI-TOF MS appears to be a powerful tool for a rapid, sensitive, and differential characterization of *C.b.* isolates and is a good candidate for phyloproteomic approach. However, more *C.b.* isolates have to be analyzed to prove the advantages of this method.

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