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Surface-enhanced laser desorption ionization/time-of-flight (SELDI-TOF) mass spectrometry (MS) as a phenotypic method for rapid identification of antibiotic resistance

Lenka Dubska [a,b,*], Katerina Pilatova [a], Monika Dolejska [b], Zbynek Bortlicek [c], Tereza Frostova [a], Ivan Literak [b], Dalibor Valik [a]

[a] Department of Laboratory Medicine, Masaryk Memorial Cancer Institute, Zluty kopec 7, 656 53 Brno, Czech Republic
[b] Department of Biology and Wildlife Diseases, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, 612 42 Brno, Czech Republic
[c] Institute of Biostatistics and Analyses, Masaryk University, Kamenice 3, 625 00 Brno, Czech Republic

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ABSTRACT

Based on experiments with 10 defined strains of *Escherichia coli*, we present a new method for bacterial phenotyping using SELDI-TOF mass spectrometry. Changes in bacterial protein profiles in the context of the time of cultivation and the antibiotic environment were minimal. Proteom subprofiling may further distinguish between strains with specific susceptibility to antimicrobials. Mass spec-based methods may become common in the future of bacterial pathogen identification in clinical microbiology diagnostics.

1. Introduction

SELDI-TOF MS is a variant of matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) method used for analysis of protein mixtures. SELDI-TOF MS allows rapid analysis of protein profiles of large number of crude samples using protein arrays (Protein Chips) [1]. MS analysis can be conducted on this instrument using gold chips without chromatographic surfaces.

The determination of antimicrobial susceptibility of a clinical isolate, especially with emergence of multidrug-resistant microorganisms, is often crucial for the optimal antimicrobial therapy of infected patients. Bacterial identification using MALDI mass spectrometry was proposed 35 years ago [2], currently several studies have introduced this method as an accurate and rapid approach for bacterial identification [3,4] and bacterial mass spectra databases are expanding. However, MS profiling is still not used in routine bacteriology practice. We present mass spec-based phenotyping of *Escherichia coli* in the context of culture-time dependent variation, growth in the presence of antibiotics, and strain specificity.

2. Materials and methods

2.1. Growth of E. coli ATCC 25922 in Lauria-Bertani (LB) broth

Overnight LB broth culture was inoculated into 250 ml of fresh LB broth. Cells were grown at 37 °C under aeration in LB broth at a constant shaking rate of 135 rpm. Growth was monitored by measuring the turbidity – optical density (OD) with BioPhotometer Eppendorf 6131 photometer using wavelength 600 nm and 0.5-ml aliquot of bacterial suspension from each time point was used for MS profiling.

2.2. Cultivation of atb-resistant strains and atb-resistance typing

Strains were isolated by cultivation onto McConkey agar (MCA) containing cefotaxime (2 mg/l) to detect *E. coli* strains with extended-spectrum β-lactamase (ESBL) and subsequently on MCA supplemented with ciprofloxacin (0.05 mg/l) to isolate fluoroquinolone-resistant *E. coli*. Strains growing on MCA with cefotaxime were examined using the double-disk synergy test to confirm the production of ESBL. The strains were identified using API 10S test kit (BioMerieux, France).

The genes responsible for the ESBL phenotype (bla_{TEM}, bla_{SHV} and bla_{CTX-M}) were identified by PCR and further analyzed using

* Corresponding author. Masaryk Memorial Cancer Institute, Zluty kopec 7, 656 53 Brno, Czech Republic. Tel.: +420 543 136 704.
E-mail address: dubska@mou.cz (L. Dubska).

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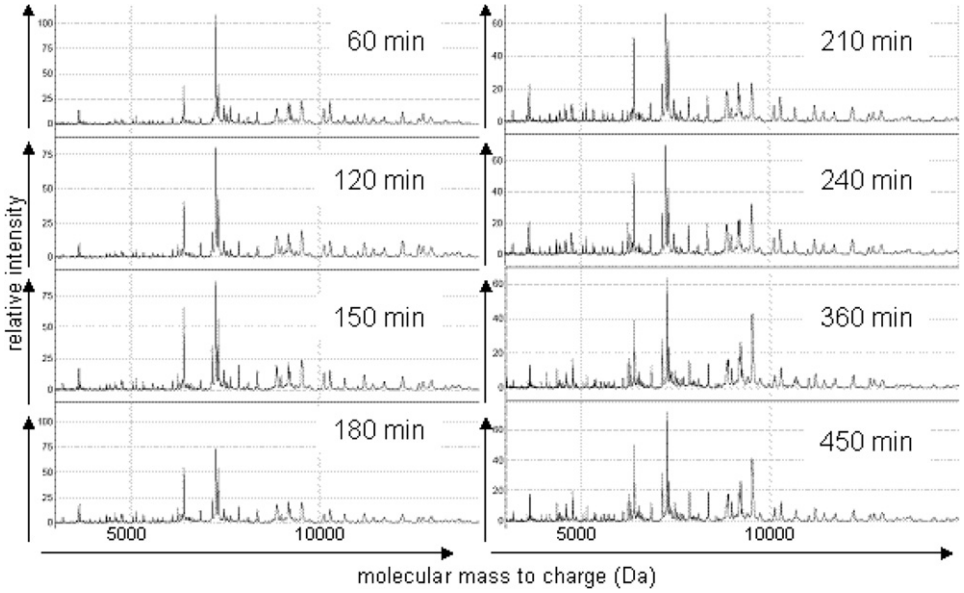

Fig. 1. Growth-dependent variation in MS spectra of *E. coli* strain ATCC 25922. Protein spectra from various time points of cultivation.

sequencing. The colonies grown on MCA with ciprofloxacin were tested for plasmid-encoded quinolone-resistance genes *qnrA, B, C, D, S*, *qepA* and *aac(6′)-Ib* by PCR. Susceptibility to additional antimicrobial agents was tested by disk diffusion method in accordance with the Clinical and Laboratory Standards Institute. PCR amplification was used to detect specific antibiotic resistance genes (*tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *tetG*, *cat*, *cmlA*, *floR*, *sul1*, *sul2*, *sul3*, *strA*), integrase genes *int*1 and *int2*, and gene cassettes (*dhfr1*, *dhfr12*, *dhfr17*, *aadA1*, *aadA2*, *aadA5*, *estX*, *sat1|2*) within class 1 and 2 integrons.

For MS analysis, strains stored at −80 °C were cultivated on LB agar without antibiotics and subsequently on LB agar with cefotaxime (2 mg/l) or ciprofloxacin (0.05 mg/) depending to their antibiotic resistance phenotype.

2.3. *E. coli* cultures processing for SELDI-TOF MS

One colony was suspended in 100 μl of distilled water, frozen at −70 °C and thawed. Expression difference mapping on ProtenChip arrays was carried out using gold chips (Bio-Rad and Ciphergen Biosystems) for MALDI analysis using SELDI-TOF mass spectrometer. One μl of bacterial suspension was applied on a gold chip, overlaid two times with sinapinic acid matrix (saturated solution of 5 mg sinapinic acid in 400 μl 0.5% trifluoroacetic acid and 50% acetonitrile) and allowed to dry at 30 °C for 30 min after sample and matrix applications. Samples were prepared for analysis in triplicates.

Measurement was performed on the ProteinBiology Sytem II (PBSII) (Ciphergen Biosystems) and processed with the ProteinChip Software Biomarker Edition, Version 3.2.1 (Ciphergen Biosystems). After baseline subtraction, spectra were normalized by a total ion current method and peak detection was performed using Biomarker Wizard software (Ciphergen Biosystems) using signal-to-noise ratio of 3.

2.4. Statistical methods

Analysis was performed based on 150 peaks per each spectrum within the *m*/*z* from 3 to 20 kDa. In order to find similarities between various strains and conditions, the normalized and aligned spectra were clustered using Diana divisive hierarchical algorithm with Euclidean distance as a measure of dissimilarity. The result of clustering is plotted in Fig. 3. The analysis was performed using *R* – the software for statistical computing, v.2.8.1. [5].

3. Results

3.1. Growth phase-dependent variation in MS profiles

To assess growth-dependent changes in the pattern of MS profiles, we analyzed cell aliquots removed from continuous broth cultivation. We observed that protein spectra are more complex at the end of exponential phase (210–240 min) and remain unchanged in stationary phase (Fig. 1).

3.2. Analysis of defined atb-resistant strains of E. coli

MS profiles were obtained from *E. coli* strains with genotypically and phenotypically defined resistance to antibiotics and from *E. coli* strain ATCC 25922 (Table 1). Moreover, analysis involved antibiotic grown counterparts from resistant strains to evaluate the influence of the antibiotic environment on the protein composition. Analysis was performed in triplicates. We obtained over 50 peaks that were

Table 1
Defined and tested 10 *E. coli* strains: collection strain from ATCC and 9 strains with characterized antibiotic resistance phenotype and genotype.

Isolate no.	Atb-resistance phenotype[a]	Atb-resistance genes
1PL	AAcCfCipNa	$bla_{CTX\text{-}M\text{-}1}$
5PL	ACfSSuSxtT	$bla_{CTX\text{-}M\text{-}1}$, *strA*, *aadA1*, *sul2*, *tetB*
78PL	ACCfSSuSxtT	$bla_{CTX\text{-}M\text{-}1}$, *cat*, *strA*, *sul1*, *sul2*, *tetB*, *int1*, I1 − 2.5 kb: *dhfr1-aadA1*
88PL	ACf	$bla_{SHV\text{-}12}$, *int1*, I1 − 2.1 kb: *aadA2*
N8	ACfCipNaSSuSxtT	$bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, *aac(6)-Ib-cr*, *sul1*, *tetA*, *int1*, I1 − 1.7 kb: *dhfr17-aadA5*
18PL	ACipNaT	bla_{TEM}, *tetA*, *int1*, *qnrS*
58PL	ACipNaSSuSxtT	bla_{TEM}, *strA*, *aadA1*, *sul2*, *tetA*, *int1*, *qnrS*
1LA	ANaSSuT	bla_{TEM}, *sul1*, *sul2*, *tetA*, *int1*, I1 − 1.0 kb: *aadA1*, *qnrS*
1	AST	bla_{TEM}, *strA*, *tetA*, *qnrS*

[a] A, ampicillin, Ac amoxicillin-clavulanic acid, C, chloramphenicol, Cf, cephalotin, Cip, ciprofloxacin, Na, nalidixic acid, S, streptomycin, Su, sulphonamides, Sxt, sulphamethoxazole-trimethoprim, T, tetracycline.