Proteomic analysis of two *E. coli* samples and sequencing of acetylase mediated from the pMdT1 recombinant plasmid

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Introduction

Escherichia coli is a commensal microorganism of the gastrointestinal tract of animals and humans [1]. The overuse of antibiotics in different areas such as human and veterinary medicine, coupled with different mechanisms of gene transfer (such as horizontal gene transfer) may have contributed to the spread of antibiotic resistance, a major public health problem. The mechanisms of gene transfer allows us to understand the acquisition of resistance mechanisms in different organisms [2,3]. The presence of pMdT1 plasmid containing a gene that encodes a variant of the AAC (6')-lb-cr protein which confers resistance to kanamycin and tobramycin, and decreases the susceptibility to ciprofloxacin and norfloxacin, has great importance in the study of antibiotic resistance [4].

The aim of this study was to characterize the total proteome of two *E. coli* strains (*E. coli* Electromax DH10B and *E. coli* TF-Se20) by two-dimensional electrophoresis (2DE) according their isoelectric point and molecular weight (IEF x SDS PAGE) followed by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/MS) identification. Finally the protein of interest by liquid chromatography coupled to mass spectrometry was sequencing.

Material and Methods

Two *E. coli* samples were analysed. Electromax DH10B is a transformation-ready strain and TF-Se20 is a strain that contains the pMdT1 plasmid expressing the acetylase gene. The workflow representing the experimetal design conceived was presented in Figure 1.

Bacterial growth conditions
- BHI agar and BHI broth

Whole-cell protein extraction

Protein Quantification
- 2D Quant Kit

Two-dimensional electrophoresis
- IEF x SDS-PAGE
- Separation by molecular weight and pI

MALDI-TOF/MS
- *Peptide Mass Fingerprinting*

LC-MS/MS
- Sequencing of acetylase

Figure 1. Workflow representing the experimental design conceived for proteomics approach.

Results and Discussion

After IEF x SDS-PAGE and spot excision (Figure 2) and subsequent analysis by MALDI-TOF/MS it was possible to identify 76 distinct proteins on the TF-Se20 strain, whereas 71 had a known function. From Electromax DH10B strain 72 different proteins were identified of which 71 were a biological process associated. Biological processes such as the molecules biosynthesis, glycolysis, protein biosynthesis, translation and transcription were the most representative in both strains (Figure 3).

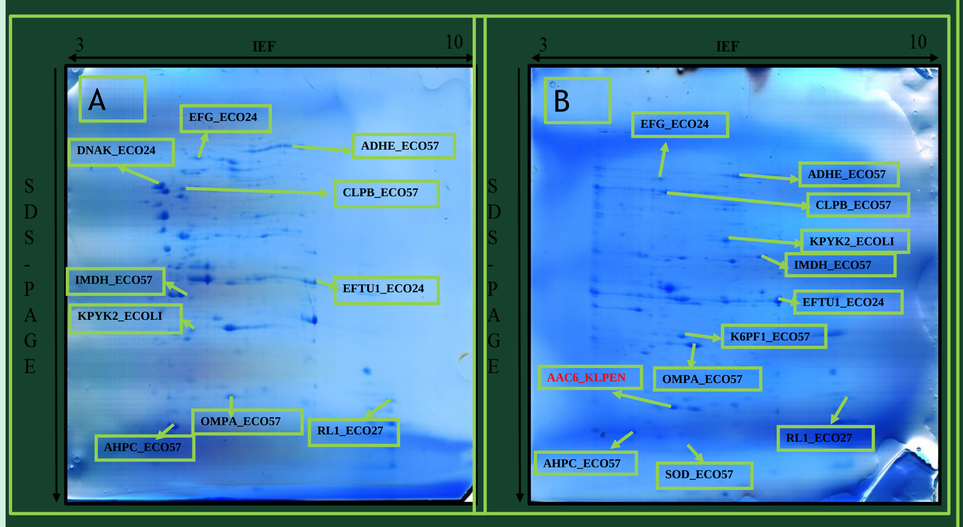

Figure 2. A. Two-dimensional gel of *Escherichia coli* DH10B stained with Coomassie Blue G-250. B. Two-dimensional gel of *Escherichia coli* TF-SE20 stained with Coomassie Blue G-250. The most relevant identified proteins are highlighted in green boxes.

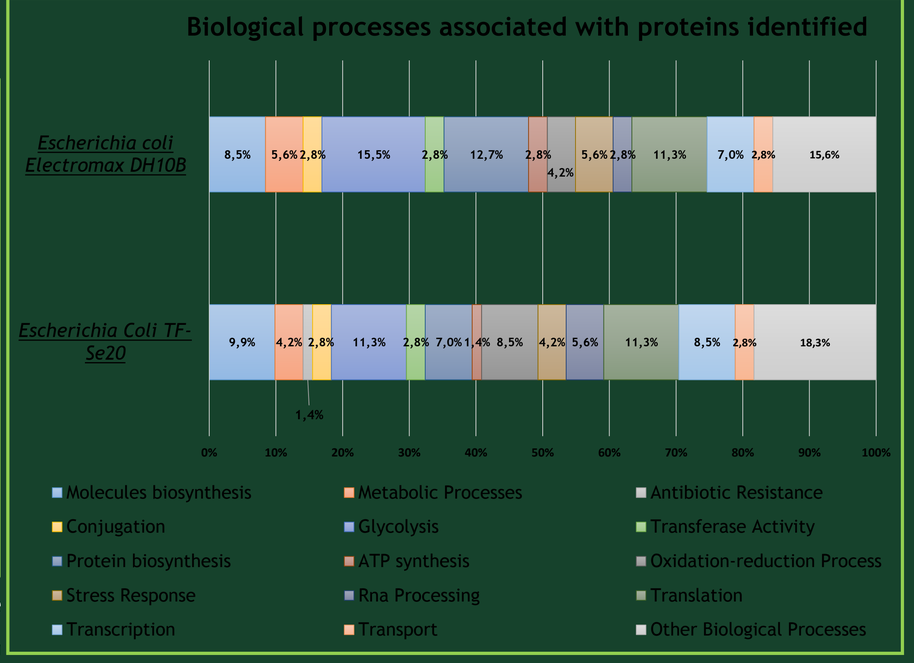

Figure 3. Biological processes associated with proteins identified in *Escherichia coli* Electromax DH10B and E*scherichia coli* TF-SE20.

The protein of interest, aminoglycoside N (6')-acetyltransferase type 1, was identified by MALDI-TOF/MS (Table 1) and in order to determine the sequence was performed liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). This protein was identified only in the TF-Se20 strain and was the single protein associated to antibiotic resistance. The results of LC-M/MS were analysed through the Mascot and Peaks search engines, allowing the identification of a protein AAC with six peptides. Overall, about 75% of the sequence was covered as shown in Figure 4.

Table 1. Aminoglycoside C (6') - acetyltransferase type 1 identified by MALDI-TOF/MS.

Spot	Acession number	Protein name	Species	Gene name	Protein MW (Da)	pI value	Mascot score	MS coverage	Biological process
6	AAC6_KLEPN	Aminoglycoside N(6')-acetyltransferase type 1	Klebsiella pneumoniae	aacA4	22450	4.91	73	30	Antibiotic resistance

1 MSNAKTKLGI TKYSIVTNS(T) DSVTLRLMTE HDLAMLYEWL NRSHIVEWWG

51 GEEARPTLAD VQEQYLPSVL AQESVTPYIA MLNGEPIGYA QSYVALGSGD

101 GRWEEETDPG VRGIDQLLAN ASQLGKGLGT KLVRALVELL FNDPEVTKIQ

151 TDPSPSNLRA IRCYEKAGFE (G)QGTVTTPYG PAVYMVQTRQ AFERTRSDA

Figure 4. Sequencing of aminoglycoside N(6')-acetyltransferase type 1 protein using LC-MS/MS.

- Peptide YSIVTNS (T) DSVTLR showed a substitution mutation of asparagine with threonine;
- Peptide SHIVEWWGGEEARPTLAD VQE had a match with Mascot only (classified as "possible but uncertain");
- Peptide IA MLNGEPIGYA QSYVALGSGDGR was confirmed and validated several times, both as a whole and in one piece.
- Peptides GLGTKLVR, MSNAKTKLGITK and QAFERTRSDA were not sequenced correctly.
- Peptide CYEKAGFE (G)QGTVTTPYG PAVYMVQTR was validated by Mascot, presenting a mutation in which glycine replaced arginine.

With BLAST tool it was established that the protein sequence belongs to two super families, N-acetyltransferase (NAT, N-acyltransferase) and AlcB (Figure 5 A). The multi-domain protein aminoglycoside-6'-N-acetyltransferase type Ib belongs to AacA4 family. Aminoglycoside-6'-N-acetyltransferase is an enzyme that modifies and invalidates aminoglycoside antibiotics such as kanamycin and tobramycin. BLAST was also did between the sequence obtained by LC-MS/MS (query sequence) and sequence protein (AAC6_KLEPN) contained in the *UniProt* database (Figure 5 B), when this alignment obtained a score of 386 and 95% identity (189 amino acids in correspondence 199).

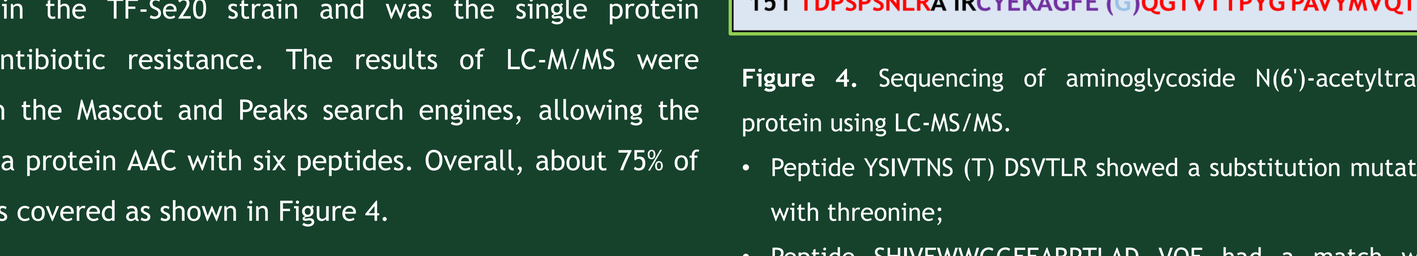

Figure 5. A. BLAST of the sequence obtained by LC-MS/MS; B. BLAST the sequence obtained by LC-MS / MS and the protein of interest identified by MALDI-TOF/MS- Aminoglycoside N (6')-acetyltransferase type 1 (AAC6_KLEPN). The differences between the two sequences are highlighted in red. (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Conclusion

The application of proteomics helped to clarify and to obtain more information about the mechanisms of resistance. The comparison between the proteomes as well as getting the biological processes of *E. coli* TF-SE20 and *E. coli* Electromax DH10B strains was carried out. Finally this approach was revealed essential to obtain analyse the sequence of acetylase mediated by pMdT1 plasmid.

References

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