PROTEOMIC STUDY OF ANTIBIOTIC RESISTANCE IN ESCHERICHIA COLI STRAINS

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Enteropathogen *Escherichia coli* infection is the most common type of colibacillosis of young animals (primarily pigs and calves), and it is cause of diarrhoea among travellers and children in the developing world. The main virulence attributes of pathogens Escherichia coli are adhesins and enterotoxins, which are mostly regulated on large plasmids. In the current study, comparative proteomics was applied to identify changes in proteins responsible for antibiotic resistance in different in vivo isolates Escherichia coli. In particular it has been studied strains with same virulence factors, but a completely different antibiotic profile, obtained from different organs of the same animal.

Key words: *Escherichia coli* enteropathogen, antibiotic resistance, proteomics

Abbreviations: 2-DE, two dimensional electrophoresis; IPG, immobilized pH gradient; SDSPAGE, sodium dodecylsulphate polyacrilammide gel electrophoresis

Introduction

Escherichia coli infections are frequently cause of gastrointestinal disease both in human and animals (1). These pathologies are classified as zoonosis (2) and the transfer to human host happens through the consumption of not pasteurized food, raw meat, or contaminated water. Despite the pathogenesis mechanism is often well known, the treatments for the Escherichia coli enteritis results often inefficient because of the antibiotic resistance. The evaluation of protein profiles in respons to various mechanisms of stress, such as, the sensitivity to antibiotics or the modification related to the antibiotic resistance (3), could be represent a valid and integrating approach for the development of new therapeutic strategies.

Bacterial surface proteins are important for the host-pathogen interaction and they are frequently involved in disease pathogenesis. A wide variety of bacterial surface proteins is represented by lipoproteins which are important components of transport system, transmembrane structures involved in the import-export of substrates, including sugars, amino acids, oligopeptides, polyamines, various metal ions and minerals. These systems contribute to many bacterial processes, such as acquisition of nutrients, stress responses and intercellular signalling, many of which could be vital for bacterial growth and survival within the host.

Proteomics represents a new molecular approach to study bacteria and infectious disease, and it is a valid instrument to analyze molecular mechanisms involved in antibiotic resistance and to evaluate new diagnostic strategies. Therefore, in this work, gel-based proteomics has been used, to study protein expression in *Escherichia coli* strains with different antibiotic-resistance profiles.

Material and methods

Escherichia coli strains were characterized for virulence and antibiotic resistance, therefore it has been selected pathotypes with same virulence factors, but different antibiotic profile. Cellular pellet for 2-DE analysis were solubilised in lysis buffer (9 M urea, 4% CHAPS, 1% DTT, 15 mM TRIS) and disrupted by sonication 5 times for 4 min at maximum power. After removal cell debris by centrifugation, proteins were precipitated (4) to minimize contamination by lipids, phospholipids and other cellular components using a solution consisting of tri-n-butyl phosphate: acetone: methanol (1:12:1). Cellular pellet was then resuspended in lysis buffer and the protein concentration was detected using 2-D Quant Kit (GE Healthcare).

Home made IPG strips (13 and 18 cm) with a linear pH range of 3.5-10 and 4.0-5.5 (5) were rehydrated overnight using a specific buffer. The amount of 40 µg (silver staining) and 400 µg (coomassie staining) of protein samples were loaded into IPG strips via cathodic cup loading. Isoelectric focusing was applied using Ettan IPGphor IEF system (GE Healthcare) at 20°C with a current of 160 µA/strip. After equilibration of strips, SDSPAGE was performed on 10% polyacrylamide gels. After runs, gels were stained with colloidal coomassie (Fig.1) and silver stain and digitalized with Pharos FX Plus Laser Imaging System (BioRad).

Image were analyzed with specific softwares to evaluate both quantitative and qualitative expression profile changes (fig.2). The raw images were processed and altered spots were compared on their volume percentages in the total spot volume over the whole gel image.

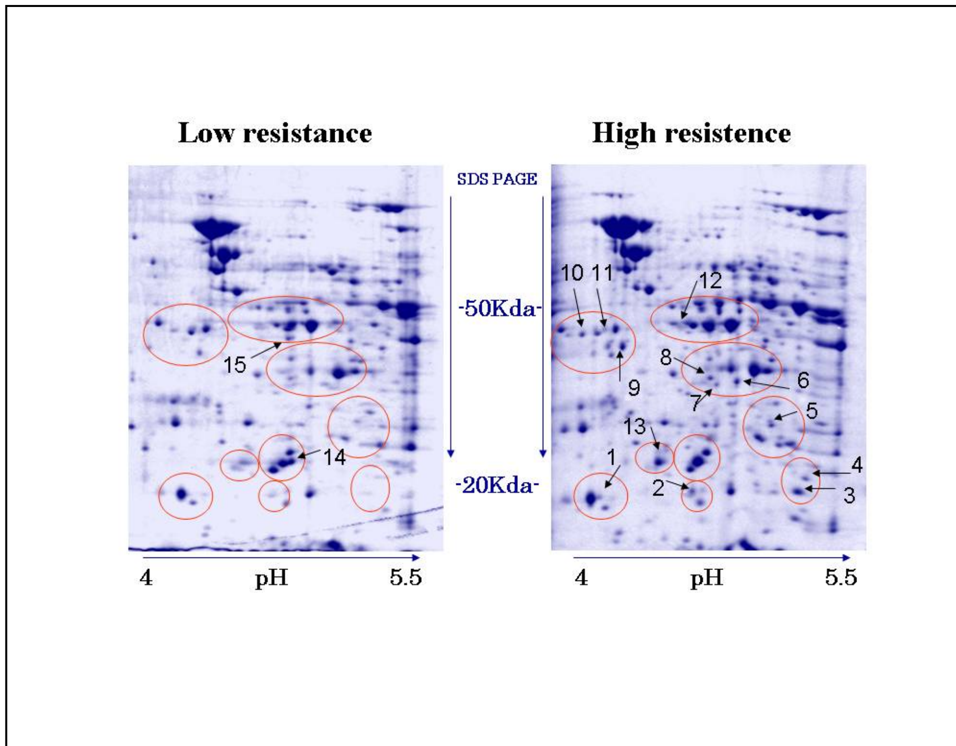

Fig.1: 2D maps, pH 4.5.5, coomassie stain. In red circles main spot differences are highlighted.

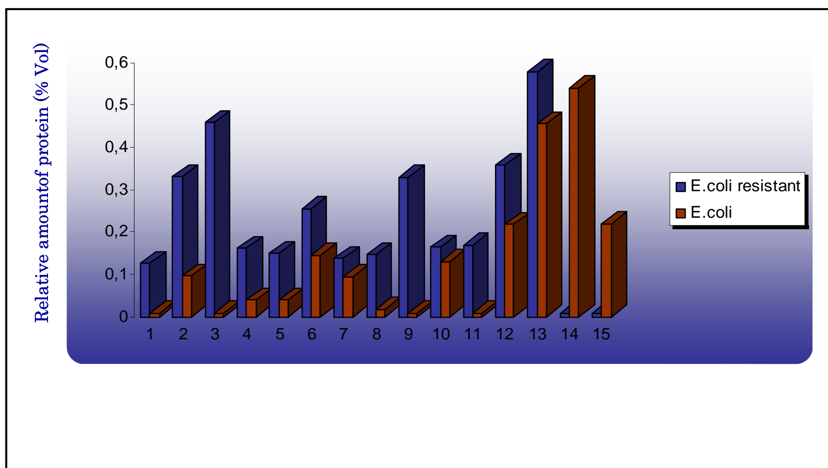

Fig.2: quantitative expression profile changes

Results and discussion

It has been carried out a comparative analysis between strains highly resistant to all antibiotics, except for penicillin, and strains resistant only to some classes of antibiotics (E.coli 111).

Gels were matched with reference Escherichia coli maps (EcoProDB, http://eecoli.kaist.ac.kr) for a preliminary identification of proteins. A first comparison show that glycolitic enzymes do not change remarkably, while there are

some differences in proteins like peptidase, (COG0693: Putative intracellular protease/amidase) catalytic proteins, (agmatinase) stress proteins, (alkyl hydroperoxide reductase, with antioxidant activity), or stringent starvation protein A, that takes part in the duplication of DNA (6). These proteins should be correlated with stress conditions such as particularly antibiotics resistance and could be potential targets for designing new drugs to inhibit the growth of the antibiotic-resistant bacteria.

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