Proteomic Analysis Of Escherichia coli Associated with Urinary Tract Infections

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Introduction

- Gram-negative bacteria is a major cause of urinary tract infections (UTI) and particularly Escherichia coli (E. coli), which is the causative agent of 80-90% of community-acquired infections, approximately 40% of nosocomial UTIs and 25% of recurrent infections. Proteomic is used to analyze and identify changes in protein profiles as biomarkers in pathological setting and can similarly be used to identify protein fingerprints for bacterial infections; proteins such as haemolsyin and adhesion protein P are highly expressed in different strains of E. coli. Here, we compared protein profiles of E. coli from different UTI patients to identify possible unique protein signatures as biomarker candidates for future studies.

- Protein from bacterial pellets cultured from urines samples from seven E. coli-associated UTI patients were extracted by sonication, separated using 1D- and 2D-gel electrophoresis, analysed using liquid chromatography mass spectrometry LC/MS, then matched against a known protein database.

- Many differences were observed in protein profiles of E. coli isolates in both 1D SDS-PAGE and 2DGE. Two bacterial proteins identified as possible candidate biomarkers were membrane protein A (OmpA) found in gram negative bacteria and RNA polymerase-binding transcription factor DksA mostly found in E. coli.

- Proteomics can be used to identify unique proteins that can be part of a standard proteomic profile as possible biomarkers for E. coli-associated UTI. We identified two such proteins, OmpA and DksA, as unique candidate biomarkers for further investigation.

Materials and Methods:

Seven urines samples were taken from different UTI patients. The pathogenic agent among those patients was E. coli. All samples were cultured on Colombian agars and incubated overnight aerobically at 37°C for proteins extraction preparations. After the overnight incubation, the plates were inspected for growth.

E. coli isolates were then loaded onto SDS-PAGE for separation. Then, E. coli isolates with abundant protein profile on SDS-PAGE, were selected for run on 2DGE. After 2DGE, Gels were compared to each other by using software analysis. Then, interesting spots were identified by LC-MS/MS. In addition, all samples isolates were checked by Bruker Daltonik MALDI Biotyper Classification Results (BDMBCR) to confirm presence of E. coli.

Results:

1. Proteins profiles of all seven urine samples show some differences in proteins expression: depicted by arrows and circles.

2. 2DGE analysis show differences in proteins expression as analyzed by Progenesis SameSpot:

Spot 1128 is present in all samples apart from sample 277 except samples 7 and 14

Spot 1346 is only present in sample 15

3. Spots identification:

- Spots were identified by LC-MS/MS;
- Spots 1128 and 1346: were identified as (OmpA), membrane proteins.

Discussion:

- In this study, proteomic profiles of E. coli strains isolated from seven urine samples and separated by 1D SDS-PAGE and 2DGE and compared to look for differential patterns of protein synthesis.

- Many significant differences were observed in protein profiles of E. coli isolates in both 1D SDS-PAGE and 2DGE regardless of type of E. coli species. The reasons behaid that are might due to: different sample sources, technical errors, genetic diversities among patient, different genders, differences in age among patients and therefor the differences in strength of body’s immunity among patients, location of infection, different species of E. coli, different length of incubation periods.

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