The Andhra Agric. J 55(4):499-501, 2008

Protein Profiling of *Escherichia coli* in response to Carbon source variation

J Pramoda Kumari and G Mohan Naidu
Assistant Professor, Dept. of Microbiology, S.V.University, P.G.Centre, Kavali – 524 201

ABSTRACT

The response of *E.coli* to carbon source variation is well understood and it is commonly used as model biological system when validating an analytical method. We have characterized the proteins isolated from *E.coli* grown in glucose, lactose and acetate by using two dimensional polyacrylamide gel electrophoresis (2D-PAGE). The quantitative results obtained from our study (unpublished data) were comparable to other existing protein profiling and transcriptional profiling approaches.

Key words : Carbon metabolism, *E.coli,* Protein profiles.

Escherichia coli is a microbial symbiont found in the colon and large intestine of most warm blooded animals, which plays a critical role in vertebrate anabolism and catabolism. The environment in which *E. coli* lives is subject to rapid changes in the availability of the carbon and nitrogen compounds necessary to provide its energy and primary building blocks (Blattner *et al.,* 1997). *E. coli* survival hinges on the ability to successfully control the expression of genes coding for enzymes and proteins required for growth in response to environmental changes. (O' Farrell, 1975). Zimmer and Co-workers (2000) demonstrated the use of this method to identify those genes in *E.coli* whose expression is activated when replacing a preferred nitrogen source with a non preferred nitrogen source. Oh and coworkers (2002) performed a similar analysis where *E. coli* were grown on different carbon sources.

This investigation involves the study of *E.coli* grown with single specific carbohydrates. This approach provides an excellent model system to study subtle differences in the microbial protein profiles because there is a controlled environment in which only one parameter is varied. Using *E.coli* to better understand metabolic pathways and characterize previously unknown proteins helps validate the methodology and could lead to the discovery of novel antibiotics when applied to related virulent microbes. The results of this study correlate well with *E.coli's* known carbon source biochemistry and molecular biology.

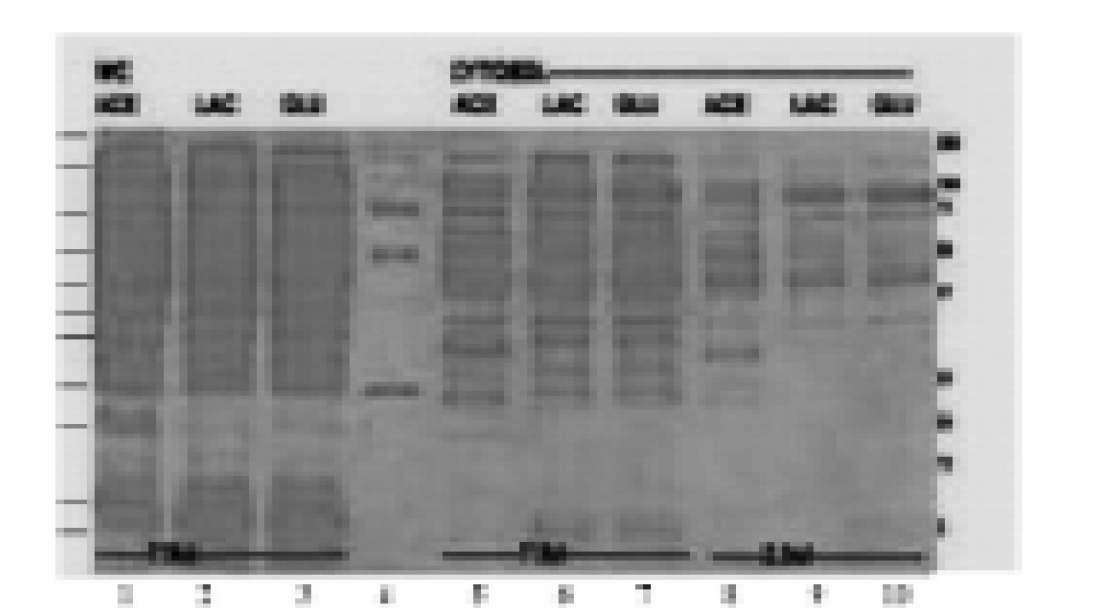
MATERIAL AND METHODS

Preparation of Media and growth conditions

Frozen *E.coli* cell stocks were streaked on to Luria-Bertani (LB) plates and grown at 37^{0}C. Individual colonies were subsequently streaked on to M9 minimal medium plates, supplemented with 0.5% sodium acetate and incubated at 37^{0}C. Seed cultures were generated by transferring single colonies into flasks of M9 minimal medium supplemented with 0.5% sodium acetate. Seed culture flasks were shaken at 250 rpm at 37^{0}C until mid log phase (OD_{600}=0.9-1.1). The seed culture was diluted 1 mL to 500 mL into separate M9 minimal media supplemented with one of the three carbon sources (0.5% glucose, 0.5% lactose or 0.5% sodium acetate). Flasks were shaken at 250 rpm at 37^{0}C until mid log phase (OD_{600}=0.9-1.1) and then harvested by centrifugation (5000g for 15 min). Culture medium was discarded and the cells were frozen at –80^{0}c until needed for protein extract preparation.

Protein extract preparation

Frozen cells were suspended in 5 mL of lyses buffer per 1 g biomass in a 50 mL falcon tube. The cells were lysed by sonication in a microson XL ultrasonic cell disrupter at 40^{0}C. The cell debris was removed by centrifugation at 15,000 g for 30 min at 4^{0}C. The resulting soluble protein extract was dispensed into 1.0 mL cryotubes and stored at –80^{0}C for subsequent analysis.



2D -PAGE analysis of protein extracts

Each protein sample was denatured and reduced using a standard PAGE loading buffer mixture containing 1.0% SDS and 10 mM DTT. The denatured protein samples were run in a Bio-Rad criterion gel apparatus (Bio-Rad) into a 12% polyacrylamide gel at 160 V for 1h. The polyacrylamide gel was stained with comassie blue using standard protocols.

RESULTS AND DISCUSSION

A standard 2D- PAGE analysis was performed on the soluble protein extracts from *E.coli* grown on three different carbon sources. The protein loading was controlled to ensure that an equal amount (30 mg) of total protein from each condition was applied on to the gel. Two aliquots (total & soluble) of total proteins were loaded to obtain better resolution of the most abundant proteins.

Figure 1:

2D-PAGE analysis of the soluble proteins generated from *E. coli* grown in minimal media with acetate (ACE), lactose (LAC) and glucose (GLU). Lane 1 to 3 describes 7.5 ml of the whole cell protein extract directly after sonication. Lane 4 contains the following protein molecular weight markers viz., 250, 150, 75, 50, 40, 25, 20, 10 and 5 K Da. Lanes 5 to 7 and lanes 8 to 10 explains the 7.5 mL and 2.5 mL of soluble protein after removing the cell debris by centrifugation respectively.

The protein profile patterns illustrated in figure 1 reveal similar patterns for the glucose and lactose growth conditions but a distinct pattern difference between the acetate growth condition and the other two growth conditions. Peng and Shimizu (2003) could be attributed to the quantitative results obtained from 2D-PAGE can be affected by proteins which exist in many spots on the gel or in instances where single spots on the gel contain many proteins. These protein profiling experiments yield important information about *E. coli's* response to environmental perturbations. Similar studies could later be generalized to investigate other biological systems. Such future studies can in turn lead to more targeted strategy to combat and detect virulent microbes, help to develop novel antibiotics and identify biomarkers for clinical discovery and diagnostics.

LITERATURE CITED

Blattner F R, Plunkett G, Blosi C A, Prna N T, Burland V, Tiley M, Collado – Vides J, Blasner J D, Rode C K, Mayhew G F, Gregor J, Davis N W, Kirkpatrics C H A., Goeden M A, Rose D J, Man B and Shao Y 1997. The complete genome sequence of *Escherichia coli K-12, Science, 277* : 1453-1474.

O' Farrell P H, 1975. High – resolution – two dimensional electrophoresis of proteins . *J. Biol. Chem. 250*: 4007-4021.

Oh M K, Rohlin L, Kao K C and Liao J C 2002. Global expression profiling of acetate grown *Escherchia coli. J. Biol. Chem. 277*:13715 – 13183.

Peng L and Shimizu K 2003. Global metabolic regulation analysis for *Escherichia coli* K-12 based on protein expression by 2 – dimensional electrophoresis and enzyme activity. *Appl. Microbiol. Biotechnol. 61* : 163-178.

Zimmer D P, Soupene E, Lee H L, Wendisch V F, Khodursky A B, Peter B J, Bender R A and Kustu S 2000. Nitrogen regulatory protein c- controlled genes of *Escherichia coli*. Scavenging as a defense against nitrogen limitation. *Proc. Nat. Acad. Sci. USA, 97* : 14674-17679.

(Received on 07.01.2008 and revised on 19.06.2008)