ELECTROPHORETIC CHARACTERIZATION OF OUTER MEMBRANE PROTEIN EXTRACT FROM ESCHERICHIA COLI ISOLATED FROM GOAT MILK IN EGYPT

*Hoda Abd El-Monem; H.S. Nada and** S. M. Nada

*Animal Health Research Institute, Dokki, Egypt

**Faculty of Vet. Med., Tanata Univ., Kafr El-Sheikh branch

ABSTRACT

Outer membrane protein (OMP) extracts of two E. coli strains O125 and O178 isolated from goat milk were purified and analyzed by use of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Each strain showed a distinct electrophoretic profile. Minor difference in profiles within strains showed the existence of different biotypes. It is concluded that SDS- PAGE may be used in the characterization and strain differentiation. The outer membrane protein extract from strain I revealed 2 distinct bands between 29.0 and 44.4 KD. On the other hand, the outer membrane protein extract for strain II revealed 3 distinct bands between the same range of molecular weight.

INTRODUCTION

Escherichia coli is one of the main etiological agents of mastitis in goats and one of the most serious causes of death losses in livestock. Several previous studies have focused on the whole cell protein. However, little is known about the outer membrane protein (OMP) of the organism. Analysis of OMP of several other gram negative pathogens has resulted in the identification of proteins that have been useful as epidemiologic and virulence markers ***(Achtman et al., 1983 and Granoff et al., 1982).***

Therefore the objective of this study reported here was to examine the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) profile of OMP- enriched extracts of 2 serotypes of *E. coliO125 and O178*.

MATERIAL AND METHODS

Bacterial strains and growth conditions:

Milk samples and blood samples were collected from a flock apparently healthy strains O125 and O178 Bacterial strain of E.coli isolated from she-goat mastitic dairy goats at Giza Governorate.

Milk samples were investigated for bacterial isolates by biochemical identification according to ***Cruickshank et al., (1975)*** and ***Quinn et al., (1994).*** *E. coli* strains were grown at 37c on Maconkey's bile salt neutral red lactose agar. The organisms were preserved on Dorset egg medium and subculture was done in soft agar pH 7.2- 7.4. blood samples were centrifuged to obtain clear sera from serological tests as shown in table 1 ***(Abdel El Ghaffar et al. 1970,Engvall 1971 and Alton et al.1988).***

Preparation of outer membrane protein (OMP) enriched extracts:

Sodium lauryl sarcosinate detergent- insoluble OMP- enriched fraction were prepared by the method of ***Barenkamp et al., (1981).*** Briefly, cells were harvested and washed twice with 0.85% NaCl and suspended in 10 mM HEPES buffer (pH 7.4), and disrupted 4 times while cooling in an ice bath, using a sonicator for 30 seconds. Intact cells and large debris were removed by centrifugation at 1700 xg for 20 minutes. The supernatant was centrifuged at 100.000 xg for 60 minutes at 4 ºC. The pellet, which contained total membrane, was resuspended in 2 ml of 2% sodium lauryl membrane. Enriched fraction was sedimented by centrifugation at 100.000 xg for 60 minutes at 4 ºC and then washed 2 times with distilled water, dialyzed against distilled water for 48 hours, lyophilized, and then stored at -20 ºC.

Sodium dodecyl sulfate – polyacy lamide gel electrophoresis:

The OMP-enriched fraction prepared from two strains of E.coli were subjected to discontinuous SDS-PAGE according the method of ***Laemmli (1970)***. Prior to loading onto the gel, the OMP- enriched fractions were heated at 100 ºC for 4 minutes in samples buffer containing 0.06 MTris, 1.2% SDS, 5 % B-mercaptoethanol, and 11.9% glycerol. Sample containing 15 µg of protein in 5o µl of samples buffer was loaded into each

lane. The protein content of the sample was determined by the modified Lowery procedure of ***Markwell et al., (1978).*** The OMP of *E. coli* was separated on SDS-polyacrylamide slab gels using Heofer mini-gel system (SE 250, Mighty small II) with PS 500 XT power supply. The completed gel used in this study consisted of a stacking and a separating gel. The stacking gel contained final concentration of 4%acrylamide/N methylene - bisacrylamide (SIGMA), 0.125 M Tris- HCl (pH 6.8) and 10% (W/V) SDS. The separation gel contained 12% acrylamide N -methylene bisacrylanide, 0.375 M Tris. HCl (pH8.8) and 10% (W/V)SDS. Polymerization was achieved by the addition of 0.05%(V/V)N,N,Ń,Ń- tetramethylenediamine (TEMED) and 0.05% (W/V)ammonium persulphate (SIGMA). The elec-trophoresis buffer (pH 8.3)consisted of 0.025 M tris base,0.192 M glycine and 0.1% SDS.

Electrophoresis was performed at room temperature at a constant voltage of 120 V until the bromophenol blue dye reached 1 cm from the bottom.Gels were stained with coomassie blue R-250. Molecular weights were estimated from a linear least-squares fit of the logarithm of molecular weight versus relative mobility of the standards. The standard error of the estimate for these plots was generally less than or equal 2± Kda.

A mixture of molecular weight standards (SIGMA chemical Co.) were prepared at a concentration of 4 mg/ml in SDS sample buffer,and 10 µl were applied to the gel. The standards were: bovine serum albumin (64 KDa), ovalbumin (44 KDa),glyceraldehydes-3- phosphate dehydrogenase (36 KDa), carbonic anhydrase (29,KDa), trypsinogen (24KDa), Soybean tryspin inhibitor(20 KDa),β-lactalbumin(18 KDa)and lysozyme (14 KDa).

RESULTS AND DISCUSSION

SDS- PAGE analysis of major polypeptides from outer membrane of two strains of *E. coli* were visualized by coomassie staining. The protein profile of each Strain O125,O178 is represented in Fig. (1).

	1	2	3

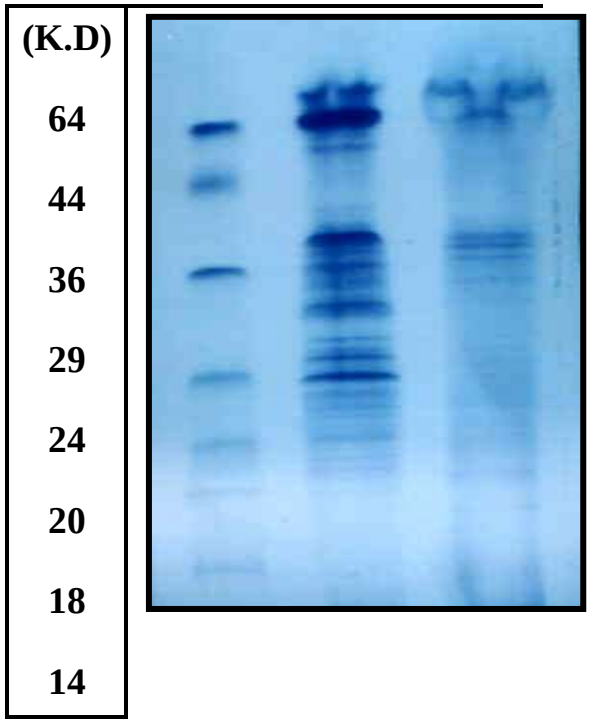

Fig. (1): SDS- PAGE analysis of outer membrane protein enriched extract of two *E. coli* strains.

1) Molecular weight marker.

2) Strain O125.

3) StrainO178.

Table (1): Serological detection for E. coli in good:

No of blood samples	Eliza test		Agglutination		Complement fixation test	
	No + Ve	%	No + Ve	%	No + Ve	%
125	7	6.5	6	4.8	5	4

The outer membrane protein extract from strain O125 (Fig.1 lane 2) revealed two distinct bands between 29 and 44 KD. On the other hand,

the outer membrane protein extract for strain O178(Fig.1 lane 3) revealed three distinct bands between the same range of molecular weight. There is a minor difference in profiles, existence of different biotypes.

In this study, a mild anionic detergent was used, sodium lauryl sarcosinate, for extraction of the OMP from *E. coli* strains. Other methods are available for extraction of OMP from gram- negative bacteria. ***Stull et al., (1985)*** compared the SDS- PAGE electrophoresis of OMP extracted from gram-negative bacteria by use of various kinds of detergents and found that the OMP obtained from Triton X-100 and lithium extractions closely approximated that isolated by use of isopykinc centrifugation.

Sodium dodecyl sulfate-polyacrylanide gel electrophoresis was used to examine the OMP profiles of 2 strains of *E. coli* isolated from goats suffered from mastitis.The protein profiles of OMP-enriched extract were remarkably similar except for minor quantitative differences.

The important aspect of this study was expressed as strain- specific OMP,which can be used as markers to identify the strains.The expression of these OMP markers was stable and reproducible.

Brogdan and Rimler(1982 and 1983)reported that the immunogen termed cross- protection factor was present in the outer membrance and that the cross-protection factor was indeed responsible for cross immunity against homologous and heterologous serotypes of *E. coli*.

Most epidemiologic and immunoprophylaxis research in *E. coli* infections have relied on serotyping for strain classification. Because serotyping does not accurately differentiate between strains, this would be useful and would provide valuable information. However, we realize that additional studies,using OMP-enriched fractions from a considerable number of isolates, need to be analyzed by SDS-PAGE before the recommendation of this technique to differentiate between the different isolates of *E. coli* that are of the same serotype.

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التوصيف الكهربى للبروتين السطحى المستخلص من الميكروب القولونى المعزول من لبن الماعز فى مصر

هدى عبد المنعم* وهشام سعد ندا و سعد ندا***

***معهد بحوث صحة الحيوان بالدقى**

****كلية الطب البيطرى – جامعة طنطا فرع كفرالشيخ**

تم إجراء التحليل للبروتين السطحى (الخارجى) لعترتين O178, o125 من الميكروب القولونى والمعزول من لبن الماعز المصابة بالتهاب الضرع ووجد ان هناك اختلافات بسيطة داخل كل عترة مما يؤكد وجود عترات مختلفة وهذا يدل على أهمية الفصل الكهربى للتوصيف والتفريق بين العترات

المختلفة حيث تبين بالنسبة للعترة الأولى وجود شريطين واضحين من عديد الببيتيد بينما فى العترة الثانية ظهرت ثلاثة شرائط من عديد البيتيد حيث كان الوزن الجزيئى لهم يتراوح بين 29.000 – 44.400 كيلو دالتون.