

Study on the extraction and charecterization of outer membrane proteins of *E. coli* from poultry

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ABSTRACT

The outer membrane proteins of avian *E. coli* play significant role in the pathogenesis of avian colibacillosis and have been identified as potent immunogen. Outer membrane proteins may be pivotal to the virulence of the organism and given their prominence on the cell surface may prove to be suitable candidate for vaccine development. The *E. coli* cultures grown under the above conditions were pelleted at 4000 rpm for 10 minute at 4°C. The pellet was washed three times in 10mM HEPES buffer and finally re-suspended in 10 mM HEPES buffer. Outer membrane proteins were extracted as per the method described by Bolin and Jensen (1987). The protein profiles of outer membrane proteins were analyzed by SDS-PAGE in vertical slab gel unit. Analysis of the OMP profiles of *E. coli* serotype O6 revealed three bands approximately of 34, 38 and 44 kDa molecular weight. Serotype O20 revealed the presence of two thick bands of 34 and 38 kDa molecular weight and faint bands of 21 kDa and 58 kDa molecular weight. Similarly O60 serotype shows the presence of two major OMP bands of approximately 38, 43 kDa molecular weight. The serotype O109 had two thick bands of 34 and 38 kDa molecular weight and 21 kDa, 29 kDa and 58 kDa molecular weight faint OMP bands.

Key words: OMP, *E.coli*, colibacillosis, SDS-PAGE.

INTRODUCTION

Therapeutic agents against complex enteropathy are of little value, because not only of high cost involved but also drug resistance problem and difficulty in achieving effective tissue concentrations besides altering, the normal gut flora.

Outer membrane proteins of pathogenic Gram negative bacteria and are often implicated as virulence factors. They are surface exposed and therefore suitable for vaccine development. Outer membrane proteins are indispensable components of bacterial cells and participate in relevant functions of the microorganisms. Changes in the outer membrane protein composition might alter antibiotic sensitivity and pathogenicity.

MATERIAL AND METHODS

Bacterial strains

Extraction of outer membrane proteins

SDS-PAGE analysis

The following serotypes O6, O20, O60 and O109 isolated from the cases of colibacillosis used in the present study for extraction of outer membrane proteins.

E. coli cultures grown in laboratory were pelleted at 4000 rpm for 10 min at 4° C. the pellet was then washed thrice in 10mM HEPES buffer. Finally resuspended in 10 mM HEPES buffer. The extraction of outer membrane proteins done as per the method described by Bolin and Jenson 1987.

SDS-PAGE analysis of outer membrane proteins

The outer membrane proteins profiles were analyzed by SDS-PAGE in vertical slab gel according to the method described by Anbalagan 1985 and Hari babu 1995.

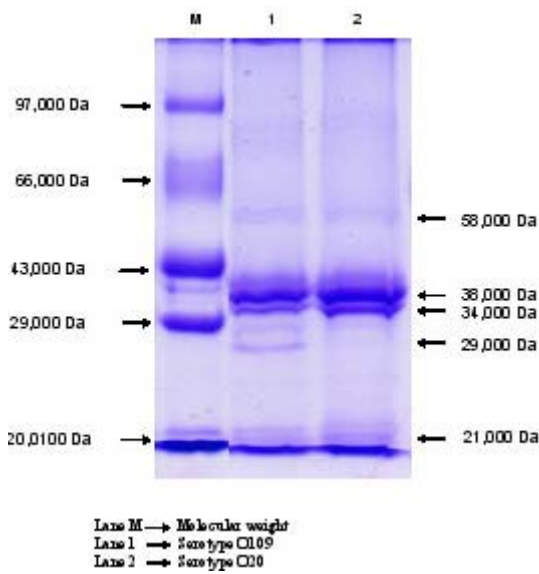


Fig. 1: SDS PAGE analysis of outer membrane proteins of serotype O109 and O20

RESULTS AND DISCUSSION

A total of seven *E. coli* isolates were recovered from fifteen processed samples, the serotype O20 and O109 were most prevalent (two each), O6 and O60 (one each).

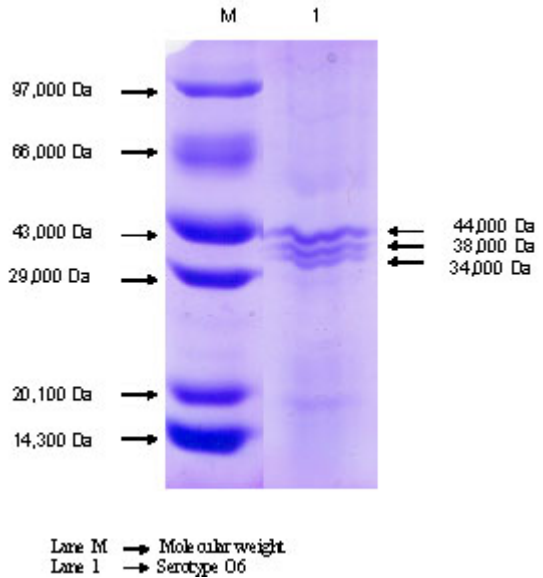


Fig. 2: SDS PAGE analysis of outer membrane protein of serotype O6

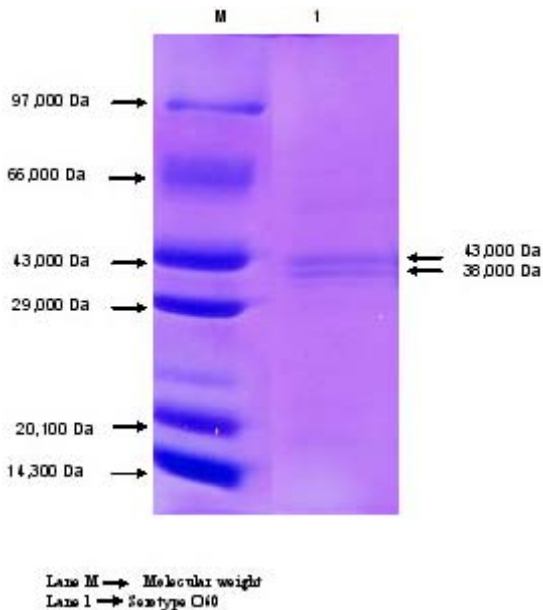


Fig. 3: SDS PAGE analysis of outer membrane protein of serotype O60

Analysis of outer membrane protein was done by SDS-PAGE. The protein profiles of OMPs revealed that the serotypes O20 and O109 had two thick bands of 34 and 38 kDa molecular weight. The 29 kDa molecular weight OMP band present only in the serotype O109. These findings correlated with the findings of Overbeeke and Lungtenberg (1980) reported that *E. coli* K12 had 2-5 OMP bands in 30-42 kDa regions.

They also contain protein bands of 21 and 58 kDa which were faint. The results are in accordance with Zadeh *et al* (2004) who observed that O2, O78 and O6 had 50, 58 and 68 kDa low density bands.

The serotype O6 revealed three bands with 34, 38 and 44 kDa. The results are in accordance with Inouye and Yee (1973) who showed the presence of 38, 44, 58 kDa by *E. coli*. Overbeeke

and Lugtenberg (1980) showed that *E. coli* K12 had 2-5 OMP bands in 30-42 kDa regions.

The serotype O60 had two bands with 38

and 43 kDa. In the present study the results were correlated with the findings of Inouye and Yee (1973) who reported the presence of 38, and 43 kDa protein bands in *E. coli*.

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