

Original Research Article

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Isolation and Protein Profiling of Outer Membrane Proteins (OMPS) of *Salmonella typhi*

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ABSTRACT

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Salmonella enterica serovar Typhi is an intracellular anaerobic gram-negative bacterium, which causes typhoid fever in humans. The emergence of multi-drug resistance *Salmonella typhi* strains has become a significant issue leading to more difficulties in the management of disease especially in developing countries. High genetic variations among the field isolates may cause vaccination failure. Therefore, there is a need of conserved and immunogenic proteins as vaccine candidate for *S. typhi*. Recent studies have shown that outer membrane proteins (Omps) of *Salmonella* have been considered as immunogens for eliciting active/protective immune response against *Salmonella* and thus, have great potential to act as possible vaccine candidates for typhoid. In present study, Outer membrane proteins of *Salmonella typhi* were isolated by ultracentrifugation method followed by quantification by Lowry method and recovery was found to be 30mg/lit approximately. Further Omps were analyzed by SDS-Polyacrylamide gel electrophoresis and nine proteins bands were observed ranging from small to medium size.

Introduction

Typhoid fever is a disease of human health concern caused by *Salmonella enterica* serovar Typhi, an important facultative gram-negative pathogen. It is a serious problem in most of the developing countries especially Southeast Asian countries, Africa and Latin America. Typhoid remains a public health

problem with an estimated 22 million cases and 200,000 related deaths occurring globally every year (Crump *et al.*, 2004). The emergence of multidrug resistance is making the situation more grimmer (Chau *et al.*, 2007). Currently available vaccines for typhoid fever have few limitations like short-term immunity, high cost, etc. and there is a need of better vaccine candidate. In last few

years outer membrane proteins of *Salmonella* have been targeted for testing of their immunopotential (Hamid and Jain, 2010; Jha *et al.*, 2015; Saxena *et al.*, 2017) as these are considered to be conserved among the field isolates. Mainly two approaches have been used by various workers. Few of workers have targeted single Outermembrane protein (Jha *et al.*, 2015; Prejit *et al.*, 2013; Saxena *et al.*, 2017) while others have used total Omeps of *Salmonella* (Hamid and Jain., 2010; Meenakshi *et al.*, 1999). In both the approaches, proteins have exhibited their immunopotential with various degree. Therefore, the present study was undertaken to isolate and characterize the Outer membrane proteins of *Salmonella* Typhi.

Materials and Methods

Bacterial strain

Pure culture of *Salmonella enterica* serovar Typhi (MTCC 733) was procured from CSIR-Institute of Microbial Technology, Chandigarh, India. The culture was revived in Luria Bertani broth and was tested by *Salmonella* specific PCR followed by biochemical characterization. Further, culture was maintained in Luria Bertani agar slants and glycerol stocks during the study.

Outer membrane proteins preparation

Outer membrane proteins of *Salmonella typhi* were isolated by method described by Choi-Kim *et al.*, (1991) with few modifications. Single colony of *S. typhi* culture was inoculated in 2 ml Luria Bertani broth and incubated at 37°C in incubator-shaker for 18 hrs. One ml of overnight culture was sub cultured in 500 ml Luria Bertani broth and incubated at 37°C for 18 hrs. in incubator-shaker. Culture was centrifuged at 10,000 rpm for 10 minutes and pellet was obtained. Further, pellet was washed twice with

Phosphate buffer saline (pH 7.2) and resuspended in 10mM HEPES buffer (pH 7.4). Culture was sonicated by ultrasonication (20 cycles (7.0μ) for 60 seconds followed by 30 seconds pause) and then centrifuged at 1700xg for 20 minutes. Supernatant was separated in a fresh tube and passed through 0.22μ filter. Filterate was ultra-centrifuged at 100,000 x g for 60 minutes at 4°C. Pellet was collected and resuspended in 2ml of 2 % Sodium lauryl sarkosinate in 10mM HEPES buffer followed by incubation at 37°C for one hour.

After incubation, cell lysate was again ultra-centrifuged at 100,000 g for 60 minutes. Supernatant was discarded and pellet was resuspended in 500 μl of Phosphate buffer saline (PBS). The protein was quantified by protein estimation method and protein profiling was determined by performing SDS-Polyacrylamide gel electrophoresis using the method of Laemmli (1970).

Quantification and profiling of Omeps of *S.typhi* (MTCC 733)

The protein was quantified by Lowry *et al.*, 1951 protein estimation method using Bovine serum albumin as a standard and protein profiling was determined by performing SDS-Polyacrylamide gel electrophoresis using the method of Laemmli (1970). The vertical slab gel electrophoresis apparatus (Atto, Japan) with glass plates of 14 x 14 cm and spacer of 1.5 mm thickness was used for performing SDS-PAGE by discontinuous buffer system using 12% resolving gel and 5% stacking gel.

Results and Discussion

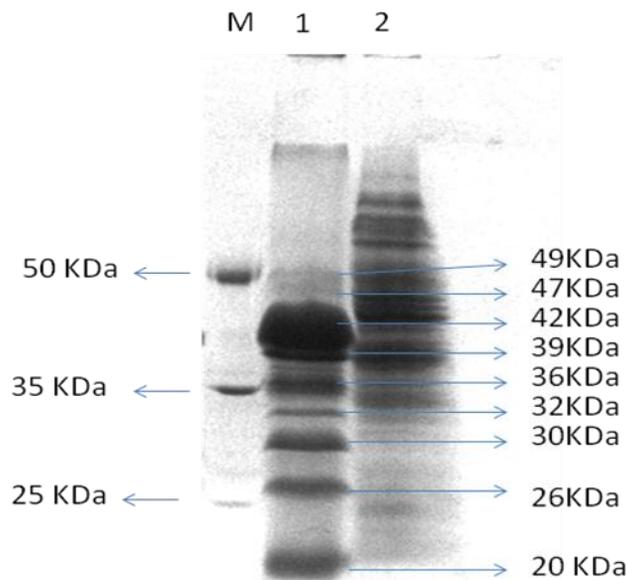
The purity of culture was confirmed by biochemical characterization and *Salmonella*-specific PCR. In biochemical characterization, culture was found to be MR+, VP-, and Urease- which is characteristic of *Salmonella typhi*.

Outer membrane proteins of *S. typhi* MTCC 733 were isolated and quantified by Lowry method and recovery was found to be 30mg/l approximately. Protein was further characterised by SDS-PAGE.

On SDS-Polyacrylamide gel electrophoresis analysis nine protein bands of approximately 20, 26, 30, 32, 36, 39, 42, 47, 49 KDa in molecular weight size were observed. Among these bands five bands of molecular weight 42, 36, 30, 26 and 20KDa were major. This showed that Omps were ranging from small to medium size, as the size of the proteins was ranging from 20-49 kDa. Our findings are very similar with findings of earlier workers as Muthiadin *et al.*, (2015) reported the Omp profile of field isolate of *Salmonella typhi* in

the same range of molecular weight as they reported the major bands of 25 KDa, 35 KDa, 55 KDa. Similarly, Kumar *et al.*, 2012 isolated and characterized the Outer membrane proteins of *S. Gallinarum* and reported the seven distinct protein bands among which 18, 38, 40, 43, 45, 50 and 60 KDa were found to be major protein bands. Though, earlier Hamid and Jain, 2008 reported the Outer membrane proteins of *S. typhimurium* ranging between 15-100 KDa, which is different from finding of other recent reports. The possible explanation is that Hamid and Jain, 2008 used different method (Foulaki *et al.*, 1989) which is composed of lysis and dialysis of bacterial protein. Later on this method was replaced by ultracentrifugation method (Choi-Kim *et al.*, 1991) (Fig. 1).

Fig.1 Isolation of Omps of *Salmonella Typhi* (M-Protein marker, 1-Outer membrane proteins of *S.Typhi*, 2- Whole cell proteins of *Salmonella Typhi*)



Salmonella is an important pathogenic organism, which causes disease in human and animals (Chui *et al.*, 2004). In human beings it causes two types of disease. Typhoid fever caused by *S. typhi* and non-typhoidal salmonellosis mainly caused by *S. typhimurium* and *S. enteritidis* (Barquist *et al.*,

2013). *Salmonella* has high morbidity and mortality rate and multiple drug resistance which have been reported from most of the part of world has made the situation more difficult (Rahman *et al.*, 2014; Kumar *et al.*, 2013; Tamuly *et al.*, 2012; Saxena *et al.*, 2004). Therefore, the vaccination is the only

viable option. Presently, available vaccines have few limitations such as they are costly and cannot be used for mass vaccination in developing countries like India (Sudharshan *et al.*, 2014). Several approaches have been used for development of suitable vaccine such as mutant vaccine (Shippy *et al.*, 2012), subunit vaccine (Hamid and Jain, 2010) and r-DNA vaccine (Saxena *et al.*, 2017). All these approaches have exhibited success with different level but still a potent protective immunogenic candidate could not be explored.

Outer membrane proteins of *Salmonella* are conserved proteins. Therefore, they can overrule the possibilities of vaccination failure due to high genetic variations (Yang *et al.*, 2013). Outer membrane proteins of several serovars have been targeted for development of r-DNA vaccine against *Salmonella* and few of them have exhibited their immunepotential such as OmpC (Jha *et al.*, 2015; Prejit *et al.*, 2013). Omp 49 (Hamid and Jain., 2010) and Omp 28 (Saxena *et al.*, 2017) but none of the single Omp could provoke complete protective immunity. Earlier in few studies, workers have used total Omps in place of single Omp and reported a better protection (Meenakshi *et al.*, 1999; Hamid and Jain., 2010). Therefore, we isolated and characterized total Omps of *Salmonella typhi*. On basis of findings of earlier workers, we can conclude as Omps are conserved proteins and if they are used along with new generation adjuvant systems like Calcium phosphate nanoparticles (Tamuly and Saxena, 2012) they may be proven as an effective candidate for vaccine development against *Salmonella Typhi*.

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