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Heterogeneous expression and functional evaluation of *in silico* characterized recombinant OmpC of *Salmonella* Typhimurium as a functional poultry vaccine to eradicate zoonotic transmission

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Salmonellosis is one of the major global health concerns leading to millions of deaths annually. The present vaccines not being up to the mark necessitate the need for the development of new generation vaccines. Outer membrane proteins (Omps) of several Gram negative bacteria have been investigated and found to be immunogenic and protective. The present study explores the potential of a major porin protein (OmpC) of *Salmonella* Typhimurium, as a vaccine candidate. The OmpC 3D structure and its potential to bind effectively with antibodies and generate humoral response was investigated using *in silico* docking, and expressed in a heterogeneous *Escherichia coli* M15 host strain. The rOmpC was purified and its immunopotential was evaluated *in vitro* by western blotting and *in vivo* in three weeks old chicks. The recombinant OmpC produced a significant humoral response and in vaccinated birds 100% survival rate was observed along with delay in the shedding of organism in droppings. These findings indicate that the rOmpC vaccination prevents mortality in chicken and lowers fecal shedding in droppings.

Key words: Outer membrane protein (Omp), Salmonellosis, *Salmonella* Typhimurium, subunit vaccines, porins, rigid-body docking.

INTRODUCTION

Salmonellosis is a major health concern affecting a large population globally. *Salmonella* enterica, the causative agent of Salmonellosis results in food borne illnesses and gastroenteritis in human beings and other mammals (Sharma, 2003). Due to its high physiological adaptability and broad host range it is difficult to devise prevention and control strategies against Salmonellosis. Although, the incidence rate of typhoidal Salmonellosis has been significantly controlled by vaccination and drugs, but non-typhoidal species continue to cause infections at a steady

rate in the last 15 years as indicated by Department of Health (2011). The major serovars contributing to the prevalence of Salmonellosis worldwide are *Salmonella* Typhimurium and *Salmonella* Enteritidis (Ekdahl et al., 2005; Laupland et al., 2010). Globally, non-typhoidal Salmonellosis results in over 1.3 billion cases and 3 million deaths annually (Pui et al., 2011), poultry products being the major source. The limitations in curbing the zoonotic transmission of such non-typhoidal serovars are a major drawback in the field which poses a risk to animal as well as human health. Therefore, it is essential to develop effective vaccination and strict biosecurity measures in such a manner that the transmission of *S. Typhimurium* through poultry is halted to break the circle of continuous infections. Hence, in this study recombinant antigenic product from *S.* Typhimurium is studied as a poultry vaccine which can strongly inhibit the zoonotic transmission.

Salmonella serovars were used to produce killed bacterins (Nicholas and Andrews, 1991) and attenuated vaccines by culturing the vaccine strain in iron-deficient medium (Woodward et al., 2002). Killed vaccines are usually less effective as they only contain surface antigens that give an incomplete protective antibody response, they fail to elicit cell-mediated immune response and they fail to elicit production of secretory immunoglobulin (sIgA) response. Attenuated vaccines have drawbacks like immune-suppression and fecal shedding in vaccinated animals (Barrow and Wallis, 2000). Moreover, the recent emergence of antibiotic resistance and reversal of pathogenicity in *Salmonella* further strengthen the requirement of an effective vaccine management of Salmonellosis. Several bacterial components like adhesive proteins, polysaccharides, lipoproteins and outer membrane proteins have been investigated as immunogens (Yap et al., 2001; Lin et al., 2001; Wolfenden et al., 2010; Bouzoubaa et al., 1987, 1989) which may be protective. Such factors which interface the cell with the environment may be efficiently used as immunogens so as to stimulate the development of protective immunity. The outer membrane proteins (Omp(s)) of *Salmonella* are considered functional candidates as they represent crucial virulence factors with a significant role in the pathobiology of gram negative bacteria and bacterial adaptation (Hamid and Jain, 2008).

Porins represent the most abundant class of Omp(s) that have been elucidated to be protective (Calderon et al., 1986; Isibasi et al., 1988) in few serovars of *Salmonella* (Prejit et al., 2013). They have important role in the maintenance of bacterial physiology aiding transport of small hydrophilic molecules (Nikaido, 1996), having a potential role as immunogens in diagnostic assays and vaccination (Hamid and Jain, 2008; Aron et al., 1993; Secundino et al., 2006; Singh and Sharma, 1999). Specific humoral and cellular immune responses are mounted against *Salmonella* Omp(s) (Aron et al., 1993; Secundino et al., 2006; Begum et al., 2008; Bhat and Jain, 2010; Prejit et al., 2013). Several outer membrane *Salmonella* proteins have been characterized, evaluated and found to be immunodominant in poultry (Wyszynska et al., 2004; Begum et al., 2008; Meenakshi et al., 1999; Nicholas and Andrews, 1991).

Outer membrane proteins when administered together were found to control fecal shedding of organisms in vaccinated birds (Meenakshi et al., 1999; Prejit et al., 2013). These porins of *Salmonella* contain T cell immunodominant antigenic regions and are involved in providing protection in typhoid fever and murine salmonellosis (Quinonez et al., 2004). OmpC, a major porin protein was found to be synthesized and incorporated into the outer membrane at both low and high osmolarity conditions (Arockiasamy and Krishnaswamy, 2000) depicting its role in both free living condition and infection. It is a good candidate to display heterologous epitopes on the cell surface (Puente et al., 1995; Arockiasamy et al., 2004a) and the detailed structural properties have been clearly elucidated B (aalaji et al., 2006; Baalaji et. al., 2006). The immunogenic potential was earlier studied and it was observed to be imunodominant and thermostable protein (Verma et al., 2009; Jailkhani et al., 2007). Hence, OmpC is considered as a potential candidate for the development of a subunit vaccine against Salmonellosis (Prejit et al., 2013). Further, OmpC-OmpF mutant was found to possess attenuated virulence (Chatfield et al., 1991) revealing its role in pathogenesis and was also observed to confer long lasting and specific humoral response against the pathogen (Secundino et al., 2006).

As the isolation of Omp(s) is a tedious task, production of recombinant proteins has been considered as an alternative improved strategy. The present study deals with the evaluation of OmpC as a vaccine candidate. Initially, 3D structure, epitope mapping and docking studies were conducted so as to evaluate the immune-potential of the protein *in silico*. The protein was further expressed in a heterogenous system, purified and analyzed by SDS-PAGE and Western Blotting for *in vivo* evaluation in model organisms. The *in vivo* studies of recombinant protein in chicken, further illustrates its role as a vaccine candidate with a need for a more detailed study.

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Abbreviations: Omp C, Outer membrane protein C; **PVDF,** polyvinylidine fluoride; **OPD,** orthophenyl diamine; **TT,** tetrathionate.

MATERIALS AND METHODS

Bacterial strains

The culture of *S. enterica* subsp. *enterica* serovar Typhimurium MTCC 3231 was procured from Institute of Microbial Technology (IMTECH, Chandigarh). The *Escherichia coli* M15 cells were used for the expression of recombinant protein.

Animals

Twenty one days old White Leghorn chicks were procured from Instructional Poultry Farm, G.B. Pant University of Agriculture and Technology, Pantnagar. All experiment were approved and in accordance with the guidelines of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). The birds were reared under hygienic condition and provided with feed and water *ad libitum*.

Verification of *ompC* clone

The *ompC* gene from *S.* Typhimurium MTCC 3231, was cloned in pJET1.2 blunt cloning vector using primers: Forward primer—5' GGATCCATGCGTATCGGCTT 3', Reverse primer—5' AAGCTTTTAGAACTGGTAAA 3' (Clone JET^{TM} PCR cloning kit, Fermentas, USA) (Jha et al., 2012) and sequenced by Ocimum Biosolutions Ltd., Hyderabad. The recombinant plasmid was purified by alkali lysis method (Sambrook and Russell, 2001), and was verified by double digestion using *Bam*HI and *Hind*III.

Expression of recombinant 38.5 kDa OmpC protein

The 1.0 kb gene for 38.5 kDa OmpC was directionally subcloned in pQE30 expression vector (Khanam et al., 2006) with the 6X His Tag. The expression construct was transformed in *E. coli* M15 host cells. Recombinant strains were maintained in Ampicillin (50 µg/ml) and Kanamycin (25 µg/ml) and induced with 1 mM isopropylthiogalactoside (IPTG) after OD_{600} reached 0.5 to 0.7 (Sambrook and Russell, 2001). The expression of recombinant protein was analyzed on SDS-PAGE and the molecular weight was determined.

Purification of recombinant 38.5 kDa protein by Ni-NTA affinity chromatography

The cell lysate was loaded onto the column of His-Select Nickel Affinity gel (Ni-NTA resin, Sigma, USA), pre-equilibrated with equilibration buffer (100 mM NaH_2PO_4, 10 mM Tris base, 8 M Urea, pH 8.0). The column was washed with 25 column volumes of wash buffer (pH 7.5), the bound fractions were eluted with 5 ml of elution buffer (pH 4.5) and analyzed by 12.5% SDS-PAGE and quantified using Lowry's method (Lowry et al., 1951).

In silico analysis of *ompC* gene sequence

The *ompC* gene sequence (Acc. No. JF896322) was characterized and the putative 3-D structure was determined. Structural analysis was conducted using EsyPred3D (Lambert et al., 2002) for determination of the 3-D structure. Jmol was used as a visualization tool. The Ramachandran plot of the sequence was predicted and the plot statistics was analyzed as suggested by Morris et al. (1992).

Rigid body docking prediction of OmpC recombinant protein with representative antibodies

The receptor antibody models were extracted from Protein Data Bank to study the potential ability of OmpC to strongly bind with the antibodies. HexServer (Macindoe et al., 2010) was used for rigid body Antigen-Antibody docking using OmpC 3D structure, with Range angles of 180° and Step sizes at 7.5. A non-specific 6D docking run was conducted using default values for all parameters. Since the macromolecules have shape and charge complementarity, shape plus electrostatic calculations were made. The server screens the possible complementarities and clusters the solutions with similar orientations for shape plus electrostatic correlations (N = 25 or N = 30) to give sharper results. The best docking predictions were visualized and the docking energy was determined to evaluate the stability of the *in silico* complexes.

Raising hyperimmune sera against total Omp(s)

The Omp(s) of *S.* Typhimurium were isolated as per the protocol described by Choi-Kim et al. (1991). Briefly, 1 L of *S.* Typhimurium culture suspension was sonicated in 10 mM HEPES buffer (pH 7.4). The suspension was centrifuged at 1,00,000 xg for 60 min at 4°C and washed in 2 ml of 2% (w/v) sodium lauryl sarcosinate. Hyperimmune serum was raised in New Zealand White rabbit. The animal was subcutaneously injected with 500 µg of Omp(s) emulsified in Freund's complete adjuvant, followed by 250 µg of protein as first and second booster at 10 and 21 days, respectively. Serum samples were collected from ear vein at seven days post final booster. Agglutination reaction was performed on a slide with a layer of 1% agarose in normal saline. Three wells were cut using a well borer at a distance of 1 cm from each other. 50 µl of total Omp(s) was added to one well, 50 µl of serum was added to the middle well and 50 µl autoclaved water was added to the last well. The slide was kept in a moist chamber for 48 h after which agglutination was observed.

Western blotting

Purified recombinant protein was electroblotted on polyvinylidine fluoride (PVDF) membrane using semi-dry method at 0.8 A/cm^2 as described by Towbin et al. (1979). The membrane was blocked with 1% BSA, incubated with Omp antiserum at 37°C for 1 h, washed and incubated again with anti-rabbit HRP conjugated antibodies at 37°C for 1 h. Further, substrate solution (50 mM Tris-HCl pH 7.6, 0.6 mg/ml 3-3' Diaminobenzidine, 0.6 µl/ml 30% H_2O_2) was added and the signal was developed and analyzed.

Immunization and humoral response in poultry

Thirty (30) white Leghorn birds, 21 days old were divided into two equal groups for immunization studies. Group I was subcutaneously injected with 100 µg saponin from quillaja bark (Sigma Aldrich) in phosphate buffer saline as control Group II was injected with 150 µg of recombinant purified protein formulated with 100 µg saponin per bird. Two injections at 0th and 21st day were followed by a booster dose on 28th day and the blood samples were collected from wing vein on 7 and 14th day post immunization. The serum samples were analyzed for antibody titre using ELISA. The OmpC protein was coated (100ng/well) into the wells of ELISA plate and kept at 4°C for overnight. After washing, 100 µl of blocking solution (2% BSA in PBS) was added and the plate was kept at 37°C for 1 h. The plates were washed thrice with washing solution (PBS containing 0.05% Tween-20). Hundred µl of serially diluted serum

was added and the plates were incubated for two hours at 37°C. After third washing 100 µl of anti-mouse HRP conjugated antibody diluted 1:3000 times was added and the plates were kept in incubation for one hour at 37°C. The substrate solution (24.3 ml of 0.1 M citric acid, 25.7 ml of 0.2 M dibasic sodium phosphate solution, 40 mg orthophenyl diamine (OPD), 0.04 ml of hydrogen peroxide and 50 ml of triple glass distilled water) was prepared during the time of incubation. The plates were washed thrice with washing solution. Then, 100 µl of OPD substrate was added and the plates were in incubated at room temperature in dark for 30 min. Then 100 µl of 1.5 N NaOH was added to each well. The OD was observed at 492 nm. Two weeks post immunization; birds were challenged with 100 µl culture containing 10^9 cfu of *S.* Typhimurium 3231 culture strain intraperitoneally. The challenge strain administered intraperitoneally allows the development of clear symptoms, lethargic behavior and mortality. The birds were monitored for clinical signs, mortality and shedding of challenge strain using cloacal swab cultures for 10 days. The cloacal swabs were collected on 5^{th} and 10^{th} day post challenge, enriched in tetrathionate (TT) broth and verified on Brilliant Green Agar to estimate shedding of pathogen strain form host system.

Statistical analysis

The statistical analysis for comparison between mortality and shedding rates was performed using paired and unpaired Student's t-test and the mean titre was calculated by deriving the mean of logarithmic value of reciprocal of titre with standard error. The results of challenge studies were analyzed using Chi square test with Yate's correction.

RESULTS AND DISCUSSION

Heterologous expression of *ompC* gene

The expression cassette was verified by restriction analysis and transformed in heterologous *E. coli* M15 cells. The recombinants were screened for the expression of recombinant OmpC by analyzing mini prep cultures on SDS-PAGE.

The recombinant protein (rOmpC) was found to express effectively under the regulated control of IPTG as inducer. The induced protein appeared to be of 38.5 kDa and the expression was found to be significantly high indicating a large proportion of the protein synthesis machinery being directed for the expression of recombinant protein.

Purification and quantification of rOmpC

The rOmpC was purified using Ni-NTA affinity chromatography and recombinant protein eluted at pH 4.5 was found to be almost pure in SDS-PAGE analysis (Figure 1b). The protein yield of 30 g/L (3 g/100 ml) of bacterial culture was obtained which was estimated by Lowry's method and further concentrated by dialysis. The high expression level of the rOmpC would aid in generating immunodiagnostics and vaccines.

3D structure predictions of rOmpC protein and rigid body molecular docking with representative antibodies

The protein was predicted to have 15 antiparallal β-sheets forming a β- barrel characteristic of Gram negative bacterial porins. Five alpha helical regions were observed distributed in the protein sequence. Out of them three were found to be located on the extracellular surface of the membrane. One was localized in the hydrophillic loop region directed intracellularly. The fifth helix was found to be located within the pore of the protein. It probably contributes in providing a hydrophilic surface within the pore, for the effective transport of hydrophilic molecules. The β- barrel region of the protein constitutes the transmembrane region being hydrophobic in nature. *In silico* studies revealed pore size of ~1.25 nm (Figure 2a, b) which was slightly more than 1.1 nm as predicted by Baalaji et al. (2006), for *S.* Typhi (Morris et al., 1992). The Ramachandran plot showed the phi-psi torsion angles for all residues in the structure (except those at the chain termini) (Figure 2c). The darkest areas "core" regions (red) represented the most favourable combinations of phi-psi values (Morris et al., 1992). It was observed that 86.2% of the residues were present in the favoured regions except Tyrosine -90 and aspartic acid -273 in the fourth quadrant, although no residues were found in the disallowed regions of the plot (Supplementary 1) indicating stable and favourable protein structure. Further, it was observed that the regions in the sequence having high surface probability, have a high antigenic index and the transmembrane regions were found to be highly conserved in *Salmonella* and *E. coli* (Puente et al., 1995).

Interaction of OmpC antigen with antibody receptors of surface proteins

To study the antigenic potential of the protein *in silico*, eight antibody models against surface proteins were selected and extracted from PDB. The Docking was done using HexServer (Macindoe et al., 2010) (Table 1) and binding energies were obtained. These binding energies correlated with high stability and shape complementarities between the receptor and ligand 3D structures (Figure 3a-h). The negative values predicted the stability and strength of rigid body docking predictions. Since, the binding energy is indicative of interaction energy, Vander Waals forces and hydrophobic interactions, the conclusion was made that the antigen may be a probable ligand for such antibodies within the host system. The *in silico* energy correlations emphasize the predictive immunogenic property of the recombinant antigen and its probable interaction with B-cell receptor molecules generated during adaptive immune responses.

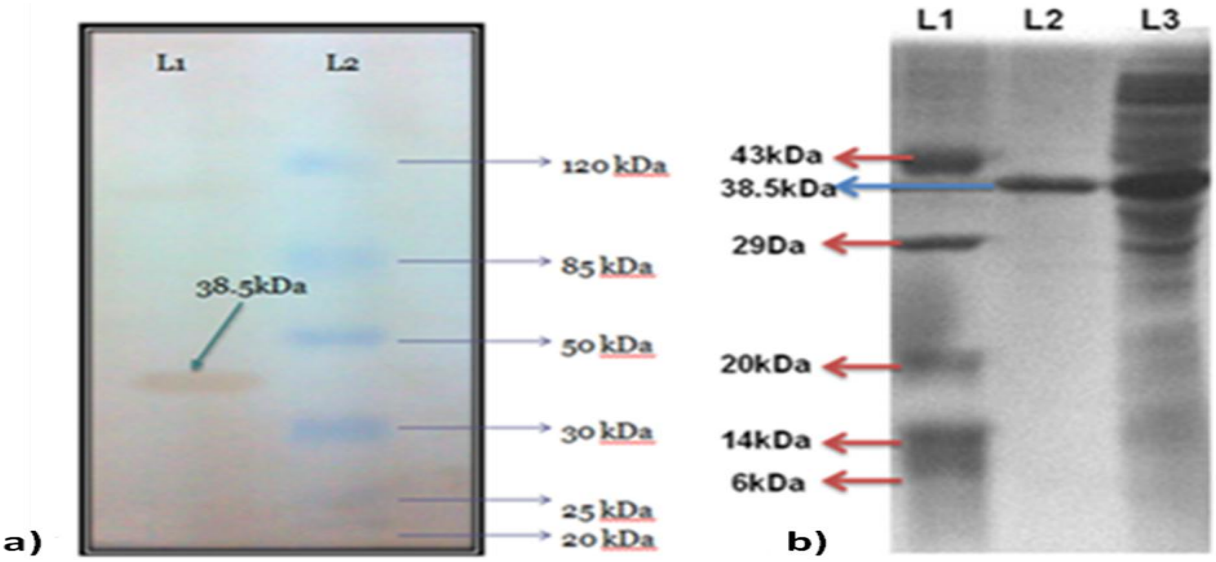

Figure 1. (a) Western blot, L1- purified OmpC protein, L2- prestained protein mol.wt. marker (Fermentas). **(b)** Purification of recombinant 38.5kDa OmpC protein by Ni-NTA affinity chromatography. L1- unstained protein mol.wt. marker (GeNei^{TM}) low range, L2- purified rOmpC protein, L3- total cell lysate.

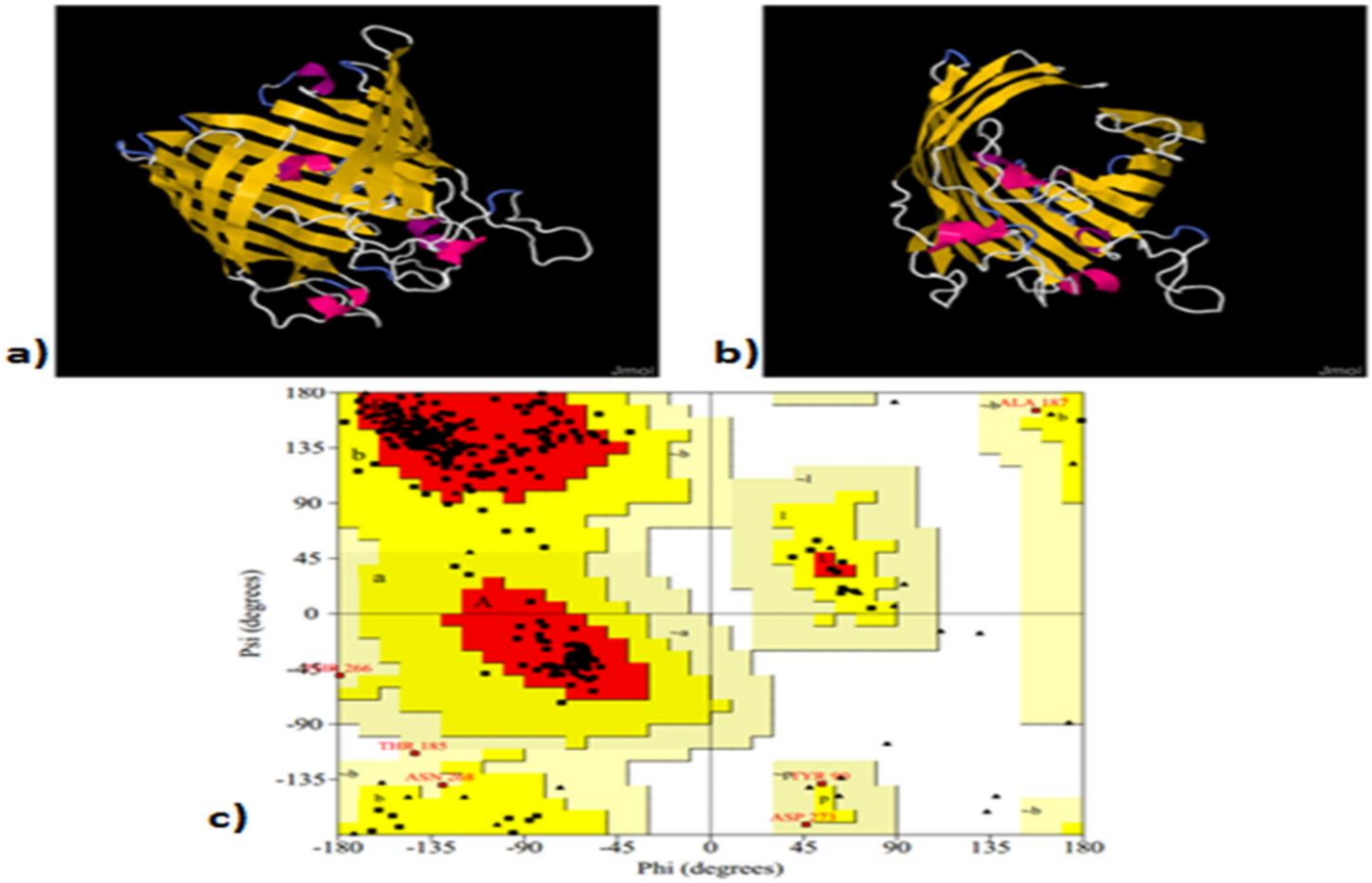

Figure 2. (a-c) The 3-Dimensional structure of OmpC porin protein predicted using EsyPred3d online resource and Jmol as visualization tool, showing major transmembrane β-sheet regions and exposed α-helical structures- front view and top view. (c) Ramachandran plot for OmpC protein generated by PBDsum for identifying the favourability of the protein.

Table 1. List of representative Receptors and their relevant interaction energies with the OmpC protein 3D structure.

PDB ID	Receptor molecule	Resolution Å	Docking energy E total (kcal/mol)
P01867 (IGG2B)	Ig gamma-2B chain C region. Igh-3	2.0	-818.31
7FAB	Crystal Structure of Human Immunoglobulin Fragment FAB. IGG1	2.0	-1296.89
1MAM	Crystal Structure of Monoclonal FAB specific for Brucella A cell wall. Igh-3	2.45	-763.87
1IGA	Model of IGA1 determined by Solution Scattering curve fitting and Homology modeling. IGH1		-8514.05
1IGY	Crystallographic structure of an intact IgG1 monoclonal antibody	3.2	-1753.32
1KCU	Crystal structure of Antibody PC287. Ig kappa chain C region	2.2	-893.53
1KCV	Crystal structure of antibody PV282.	1.8	-1167.93
3GJF	High Affinity T-cell receptor like antibodies. HLA-A, β-2-microglobulin	1.9	-853.33

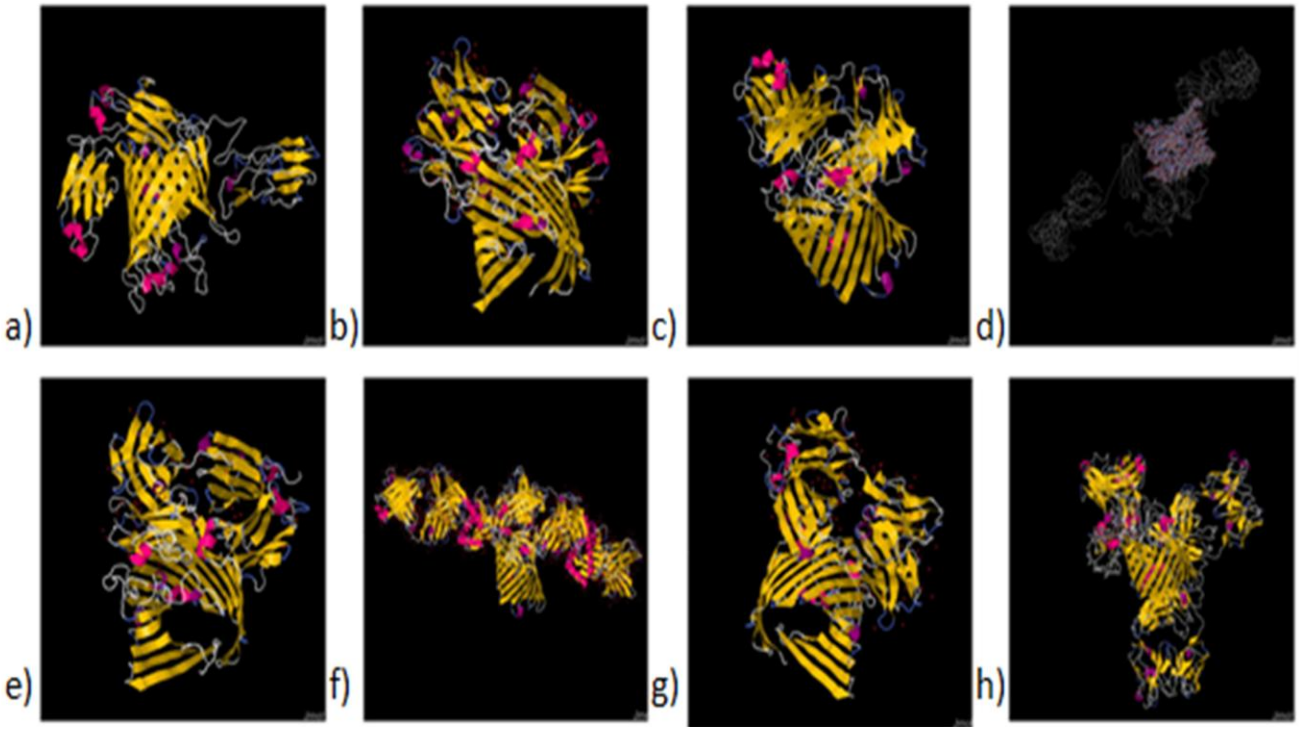

Figure 3. (a-h) Immune complex predictions using the eight 3D structures as given in Table 1.

Study of immunopotential of OmpC protein

The immunogenic potential of rOmpC was evaluated by western blotting and a sharp band was observed with anti Omp antisera indicating a strong humoral response of 38.5 kDa rOmpC in animal model as compared to total outer membrane proteins used for immunization (Figure 1a). In correspondence with the earlier *in silico* studies of *ompC* gene was done using Immune Epitope Database. The presence of many epitopes was revealed in the sequence including 13 B-cell epitopes, out of which eight were predicted to be strongly immunogenic, 14 MHC I and several MHC II epitopes (Jha et al., 2012). Further, eight major variable regions were found by multiple sequence alignment with the related sequences having high surface probability and B-cell recognition potential, indicating the high immunogenic potential of the putative protein (Jha et al., 2012). These *in silico* studies conducted earlier by us and western blot directed the animal trials for OmpC protein.

Assessment of immunogenic potential in poultry

A strong humoral immune response was elicited in immunized group. The mean of logarithm of reciprocal of

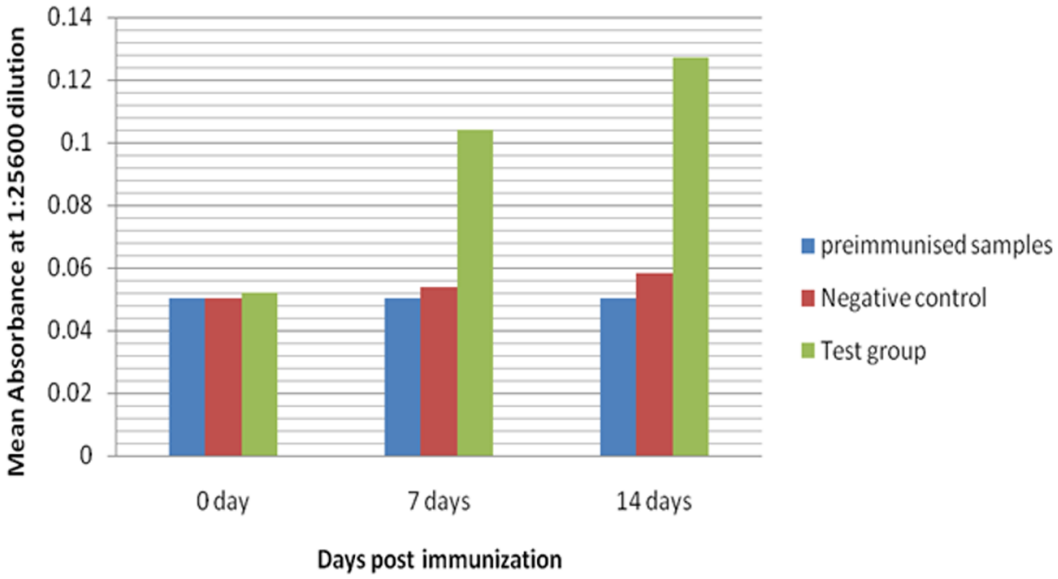

Figure 4. Comparative evaluation of recombinat OmpC antibody titres in immunized White Leghorn by ELISA, at different intervals (1:25600 dilution).

Table 2. Evaluation of the percentage of birds shedding the organism in immunized group as compared to control group.

Group	Days post challenge		
	0th day	5th day	10th day
Unimmunized control	0%	58%	83%
r-OmpC adjuvanted with saponin	0%	20%	33%

titre was estimated to be 4.40 ± 0.077 for 7th (1:29400) and 4.46 ± 0.06 for 14th day (1:32000) samples. The mean OD at a dilution of 1:25600 was calculated and the results were compared with that of control group (Figure 4). No statistically significant association was found in the humoral responses in control and vaccinated groups (p value = 0.064) revealing a strong significant antibody response generated in the vaccinated group as compared to control group.

Challenge studies

To assess the protective response generated against bacterial challenge, the immunized and control group was challenged 2 weeks post immunization. Birds in the immunized group did not show any apparent symptoms of Salmonellosis although the control group showed lethargic movements and decreased response to stimuli. In the control group, out of 15, 4 birds died at 0, 1, 3 and 4th day post challenge, although no mortality was observed in immunized group (Table 2). Cloacal swabs from all surviving birds were evaluated to estimate shedding. The *S.* Typhimurium virulent strain was identified in swabs samples using *Salmonella* specific PCR. The shedding percentage in control group was found to be 58 and 83% on 5th and 10th day, respectively, while in the case of immunized group it was 20 and 33% with no mortality. The fecal shedding of virulent strain was observed to be lower but not completely controlled in immunized birds than in unimmunized control. The association of immunization with rate of survival and with shedding was estimated by applying Chi-square test with Yate's correction. It was found that there was no significant association between immunization and the rate of survival of birds (p = 0.1071) (Supplementary 3). Similarly, no significant association was observed between immunization and shedding of bacteria (p = 0.3279) (Supplementary 4). Although, recombinant OmpC induced strong humoral response but fecal shedding of bacteria was slightly lowered and not effectively controlled. Persistence of infection was observed in some vaccinated birds. The humoral immune response developed by rOmpC immunization was found to be effective in controlling shedding in ~60% birds during challenge studies.

Porin proteins form β-barrels with long hydrophilic loops and short β-hairpin turns connecting strands on the external and periplasmic surfaces of the bilayer (Cowan and Rosenbusch, 1994). Their primary structure varies significantly among Gram-negative bacteria (Gerbl-Rieger et al., 1991), but amphiphilic β strands in the barrel are structurally conserved (Jeanteur et al., 1994). It was found that OmpC of *S.* Typhimurium folds in a similar way as OmpF of *E. coli* and OmpC of *S.* Typhi (Arockiasamy et al., 2004b; Kumar and Krishnaswamy, 2005). The

expression of OmpC during infection (Puente et al., 1989; Verma et al., 2009) and its capacity to display heterologous epitopes on the cell surface (Puente et al., 1995) make it an important candidate antigen with potential applications in immunology and vaccine design. Studies using *Salmonella* porin specific monoclonal antibodies (Muthukkaruppan et al., 1992) showed that *S.* Typhi OmpC is the major surface antigen with unique exposed epitopes. The immunogenicity of outer membrane proteins was evaluated in birds and was found to be significantly high (Meenakshi et al., 1999), indicating that systemic immunity may play a role in protection. Hamid and Jain immunized different groups of mice with selected proteins. Their ELISA results indicated that the protein elicits a significant humoral response and different levels of protection. Maximum survival was seen in the animals immunized with 49 kDa protein (100%), followed by 37 kDa (66.7%), 33 kDa (50%) and 15kDa (33.3%) (Hamid and Jain, 2008). Verma et al. (2009) evaluated the immunogenicity of the recombinant porins, in Swiss albino mice with three different adjuvants. In contrast to OmpF, the high titer ($p < 0.05$) of recombinant OmpC-specific IgG antibody was observed in mice immunized with aluminium hydroxide gel followed by Freund's adjuvant and montanide. The marked IgG responses in mice immunized with OmpC and OmpF supported the immunogenic nature of these recombinant porins and suggests that these may be used as good immunogens for vaccine studies (Verma et al., 2009). Another immunodominant surface protein of 37.81 kDa was immunologically characterized *in vivo* in chicks which induced homologous or heterologous protective efficacy against the infection of *Salmonella* serovars (Begum et al., 2008).

In the present study, although a strong humoral response was generated but the shedding of bacteria form fecal route was not completely eradicated, although significant reduction in mortality was observed. The generation of cell mediated response against *Salmonella* may be targeted in future studies by using adjuvants having depot formation property. Liposomes and ISCOMs have been reported to generate CTL induction and act as immunomodulators. These adjuvants may be used for providing the cell mediated immune response for obtaining better protection using r-Omp C as vaccine candidate.

Conclusions

Such results highlight the fact that the protein being a potent immunogen generates partially protective antibodies. These partially protective antibodies generated contribute in suppressing mortality along with slight reduction in fecal shedding of the pathogen in challenged birds. Although the antibodies may not participate in lowering zoonotic transmission, it may be hypothesized that they may play significant role in curbing mortality from virulent pathogen. Hence, it may be concluded that the protein OmpC, being immunogenic is partially protective in nature. The improvement in survival rate, suppression of physiological symptoms and reduction in fecal shedding signify the fact that the antibodies generated in the host system are partially protective. Therefore, OmpC protein may be considered as strongly immunogenic and partially protective. Further, the protective efficacy of the recombinant protein may be improved by focusing on specific epitopes and modulating the regions which improve protectivity through *in silico* experimentation. Furthermore, improvements such as new and advanced vaccine delivery systems like nano- drug delivery particles, ISCOMs, Liposomes etc which work as immunomodulators may be incorporated. These adjuvants may also be useful for providing cell mediated immune response which would further aid in enhancing the protective role of OmpC as a vaccine candidate.

Conflict of interests

The authors did not declare any conflict of interest.

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