VETERINARSKI ARHIV 88 (4), 559-568, 2018 DOI: 10.24099/vet.arhiv.170526

Cloning, expression and purification of the outer membrane protein28 of *Salmonella enterica* serovar Typhimurium for subunit vaccine development - a short communication

Mamta Pandey[1]*, Mumtesh K. Saxena[1], Anjani Saxena[1], Richa Jha[2], Sunil K. Rastogi[1], and Rajesh Kumar[3]

[1]Animal Biotechnology Center, Department of Veterinary Physiology and Biochemistry, College of Veterinary and Animal Sciences, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India

[2]College of Basic Science and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India

[3]Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India

PANDEY, M., M. K. SAXENA, A. SAXENA, R. JHA, S. K. RASTOGI, R. KUMAR: Cloning, expression and purification of the outer membrane protein28 of *Salmonella enterica* serovar Typhimurium for subunit vaccine development - a short communication. Vet. arhiv 88, 559-568, 2018.

ABSTRACT

Salmonella Typhimurium, a major gastrointestinal pathogen, poses a global threat to human health. Public health problems associated with this organism have increased to the extent that it has become a major issue. The bacterium is becoming resistant to the commonly available antibiotics, and vaccines also suffer from limitations such as short lived immunity. Therefore, there is an urgent need for the development of an effective vaccine. The outer membrane proteins (Omps) of *Salmonella* have proven their capability to be developed as a vaccine candidate for prevention of salmonellosis. With this aim, in the present study the *Omp28* gene of *Salmonella* Typhimurium was amplified, cloned and expressed under an IPTG induction system. The recombinant protein thus produced was purified and tested for its antigenicity. The antigenicity of the purified protein was confirmed by western blotting with antiserum raised in rabbit against Omps of *S.* Typhimurium. The *Omp28* gene was amplified as a 330bp product. The expressed protein was found to be of approximately 28kDa and it produced a strong signal in western blot analysis. This study concluded that Omp28 may be proven to be an effective candidate for the development of r-DNA vaccine against salmonellosis.

Key words: cloning; expression; Omp28; *Salmonella* Typhimurium; vaccine; Western blot

*Corresponding author:

Mamta Pandey, PhD, (Animal Biotechnology), Animal Biochemistry Division, National Dairy Research Institute, Karnal-132001, Haryana, India, Phone: +91 740 468 3045; E-mail: drmamtavet@gmail.com

ISSN 0372-5480
Printed in Croatia

Introduction

Salmonella is the most important gastrointestinal pathogen distributed ubiquitously. It has a broad host range and zoonotic value. Salmonellosis has often been shown to be endemic for decades in humans (JOHN, 1996), animals (VERMA et al., 2001) especially in poultry products (FAVIER et al., 2013; LOPEZ et al., 2012). MURUNGKAR et al. (2005), reported an prevalence rate of 14.40% of *S. enterica* in the north eastern region of India. The incidence of *Salmonella* in chicken meat has been reported to range from 6.79% to 97.6% using different methods (RAMYA et al., 2012). Of the total laboratory-confirmed human *Salmonella* infections, Typhimurium was found to have an incidence rate of 1.85% (CDC, 2016).

Salmonella is facultative intracellular pathogen, able to survive both extracellularly and intracellularly. Further, the rising incidence of multiple drug resistance complicate its prevention and elimination strategies. *S.* Typhimurium, being an important serovar contributing to non-typhoidal salmonellosis (LAN et al., 2016), is of major concern. It causes various illnesses, from self-limiting gastroenteritis to life threatening systemic diseases (HARAGA et al., 2008). Vaccination is an important strategy to eliminate the condition, but the available vaccines suffer from limitations, such as short-term immunity and virulence restoration. The outer membrane proteins (Omps) from *Salmonella* have been proven to have good immunogenicity (LEE et al*.,* 2010; GIL-CRUZ et al*.*, 2009). Since that discovery, *Salmonella* Omps have been characterized (HAMID and JAIN, 2008) and targeted for protection (JHA et al., 2015; SAXENA et al., 2012; BHAT and JAIN, 2010). Omp28 was isolated independently from *S.* Typhi by BHATNAGAR et al. (1982) and DE ANDRADE et al. (1998). Its complete amino acid sequence and location was deduced by NEVES-FERREIRA et al. (2004). In our previous study based on bioinformatic analysis, we proved that Omp28 of *S.* Typhimurium elicits both B and T cell mediated immunity, and has a high antigenic index (PANDEY and SAXENA, 2015). Therefore, it seemed to contain a promising vaccine candidate for poultry salmonellosis. Hence, in the present study we aimed to clone the gene, express the protein and explore its antigenicity, to prove Omp28 from *S.* Typhimurium to be an effective candidate to develop r-DNA vaccine for control of salmonellosis.

Materials and methods

Bacterial strains and growth condition. The bacterial strains used in this study were *Salmonella enterica,* subspecies *enterica,* serovar Typhimurium, strain MTCC3231 (IMTECH Chandigarh, India), *Escherichia coli* DH5α and M15 cells (Bangalore Genei, India). These cultures were stored at 4 °C in Luria Bertini (LB) agar slants and their glycerol stocks were also maintained at -20 °C. For the study, cultures were grown overnight at 37 °C in LB broth in an incubator cum shaker at 200 rpm. When required,

the above media were supplemented with ampicillin (100 µg/mL) and kanamycin (50 µg/mL) antibiotics [Himedia, India]. Antiserum against Omps of *S.* Typhimurium raised in rabbit was procured from Animal Biotechnology Centre, GBPUA&T, Pantnagar (JHA et al., 2015).

Cloning of the Salmonella Typhimurium Omp28 gene. Genomic DNA of *Salmonella* Typhimurium was isolated by the C-TAB method (WILSON, 1987). Using the available sequence information (GQ907044.1) from NCBI and GENETOOL software, oligonucleotide primers 5′GGATCCATGAATAAATTCTCCCTTGC3′ (forward) and 5′AAGCTTTTATTTTGAGAGTTCTTTCTTGA3′ (reverse) were synthesized for amplification of the *Omp28* gene. 25 µL PCR reaction mixture, containing 40 ng of genomic DNA, 20 pmole of primers, 200 µM of each dNTPs, 1.5 mM $MgCl_2$ and 2 U of jumpstart polymerase (Sigma, USA). PCR was conducted in standard conditions with the following program: initial denaturation at 96 °C for 30 sec, followed by 30 cycles of denaturation at 94 °C for 15 sec, annealing at 46 °C for 30 sec, and elongation at 68 °C for 30 sec, and a final extension at 68 °C for 5 mins. The size of the amplicon was measured by comparing it with a standard marker and it was eluted using an E.Z.N.A Gel extraction kit (Omega Bio-tek Inc.), followed by blunt end cloning in a Clone pJET™ PCR cloning kit (Fermentas, USA) in *E. coli* DH5α cells. The recombinant clones were grown in LB broth, and plasmids were isolated from them by the alkali lysis method (SAMBROOK and RUSSEL, 2001), which were then confirmed by insert release after double digestion, using *Nco*I and *Xho*I (Fermentas, USA) endonucleases.

Subcloning and expression of the Omp28 protein. pQE-30 expression vector was used for the directional subcloning of the *Omp28* gene with a 6X His tag. Using *Bam*HI and *Hind*III (Fermentas, USA) restriction endonucleases, the gene was released from the pJET 1.2/blunt cloning vector. The same restriction enzymes were also used to cut the pQE-30 expression vector. The digested insert and vector were ligated at 4 °C overnight, using T4 DNA ligase (Fermentas, USA), followed by transformation into competent *E. coli* M15 cells. Recombinants were selected on LB agar plate containing ampicillin (100 µg/mL) and kanamycin (50 µg/mL). Clones were inoculated into 3 mL of LB broth containing ampicillin (100 µg/mL) and kanamycin (50 µg/mL), and incubated at 37 °C in an incubator cum shaker at 200 rpm. The cultures were grown until they reached an OD_{600} of 0.6-0.8, that is approx. 4 hrs duration, after which cultures were induced with iso-1-propyl-thio-2-D-galactopyranoside *i.e*. IPTG to a final concentration of 1mM, and further incubated at 37 °C for 18 hrs. Expression was checked on 12.5% SDS (Sodium dodecyl sulphate) gel comparing it with the control (uninduced cells) (SAMBROOK and RUSSEL, 2001). The size of the recombinant protein was measured by comparing it with the protein molecular weight marker. Quantification of the expressed protein was done using Lowry's method (LOWRY et al., 1951).

Purification of recombinant protein. Purification of the recombinant protein was performed by the gel excision method (HAMID and JAIN, 2008). The desired protein band was removed from the gel with a scalpel blade, keeping the gel slice size to a minimum. Then the gel slice was put into a microcentrifuge tube, washed twice with triple glass distilled water, and then soaked in 1 mM DTT (Di-ThioThreitol) for 15 mins. Homogenization of the gel slice was carried out in a pestle and mortar. The required volumes of elution buffer (50 µL of 1 M DTT, 0.5 mL of 1.0 M Tris, 0.1 mL of 10% SDS, 0.3 mL of 5 M NaCl, 20 µL of 0.5 M EDTA) were added to make the final volume up to 10 mL by adding triple glass distilled water. This homogenized material was then incubated in elution buffer at 37 °C for 12 hrs in an incubator-cum-shaker, followed by a brief spin to remove the gel debris. The supernatant was removed and kept at -20 ºC until further use.

Western blotting. The purified protein, along with the marker, was run on 12.5% SDS-PAGE using a discontinuous buffer system and subsequently transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Germany) by the semi dry electroblotting method (TOWBIN et al., 1979). The transfer was carried out using a 0.8 V/cm^2 current applied for 1 hr. After that the PVDF membrane was immediately placed in blocking solution (3% BSA in PBS) and left overnight at 4 °C to block non-specific binding sites. The membrane was then washed with phosphate buffered saline (PBS) supplemented with 0.05% Tween-20 (PBST) [pH = 7.4] four times for 5 mins each, and the membrane was incubated with antisera raised in rabbit against Omps of *S.* Typhimurium at 1:50 dilution at 37 °C for 1 hr. After washing 4 times with PBST, the blot was again incubated at 37 °C for 1 hr with anti-rabbit horseradish peroxidase conjugated antibodies, at a dilution of 1:2000 in blocking buffer. The blot was washed with PBST four times for 5 mins and transferred to freshly prepared 50 mL substrate solution (Ortho Phenylene Diamine as substrate and 6 µL of H_2O_2 (30%)). The colour was allowed to develop for a few minutes and then the reaction was stopped by rinsing the blot with distilled water. The blot was dried on filter paper and stored.

Results and discussion

Salmonella Typhimurium causes one of the most common food-borne illnesses, which has been found to persist at the same rate for more than 15 years (NEWELL et al., 2010). Being highly transferable between animal and human host systems, it has been a challenging task to control the spread and transmission of the infection. Multiple drug resistance is making the situation grimmer. So, there is an emergent need for a better vaccine. The Omps of *Salmonella* have been reported to be antigenically important and have exhibited promising potential as vaccine candidates (HAMID and JAIN, 2008). One such Omp from *S.* Typhimurium, *i.e.* Omp28, was evaluated in the current study. Upon amplification the size of the *Omp28* gene from *S.* Typhimurium was found to be 330bp

(Fig. 1), which was similar to that observed by SAXENA et al. (2012) for the *Omp28* gene of *S.* Typhi. The amplified product was then cloned in pJET1.2/blunt end cloning vector and transformed in to DH5α cells. Positive clones were confirmed by colony PCR and the insert of the same size was also released by double digestion.

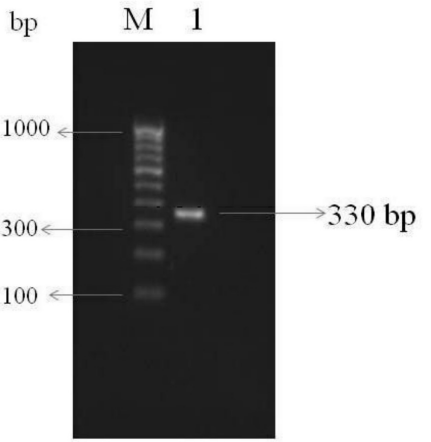

Fig. 1. PCR amplicon of *Omp28* gene of *Salmonella* Typhimurium. LaneM: Step Up™ 100bp DNA Marker (Genei™) Lane 1: PCR amplicon of *Omp28* gene

The gene was subcloned in pQE-30 expression vector and on induction with IPTG (final conc. 1 mM) the recombinant protein was expressed at 28kD. The protein was purified by the gel excision method (Fig. 2.1) and recovery of protein was found to be approximately 10 mg/L of induced culture. The purified protein was transferred onto a PVDF membrane and on western blotting, with antiserum raised against Omp(s) of *S.* Typhimurium, a very strong signal was produced (Fig. 2.2), which further confirmed the antigenicity of the Omp28 protein. Earlier some researchers reported the formation of inclusion bodies in Omps expression (MANAT et al., 2016; YOUSEFI et al., 2016), but in our study we did not observe any inclusion body formation, even at a higher inducer concentration (IPTG). Omp28 protein is formed by three identical subunits which are not linked by disulfide bonds, representing a molecular mass of 28 kDa (NEVES-FERREIRA et al., 2004). Similarly, in this study upon induction by 1 mM IPTG conc. the protein was expressed at 28 kDa in trimeric form, which probably masked the histidine tag of the protein and we were unable to purify the protein by affinity chromatography. So the protein was purified by the gel excision method. The identity of this 28 kDa protein was confirmed by western blotting, using antisera raised in rabbit against Omps of *S.* Typhimurium.

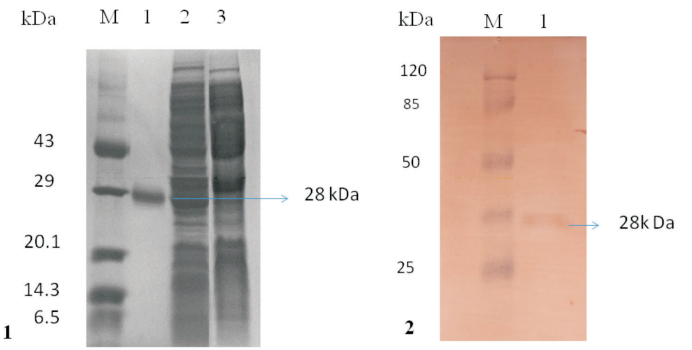

Fig. 2. 1. Purified Omp28 protein. Lane M: Protein molecular weight marker (cat no. 105978), Lane 1: Purified rOmp28 protein, Lane 2: Whole cell protein of IPTG induced pQE30-Omp28 transformed M-15 cells, Lane 3: Whole cell protein of untransformed M-15 cells. 2.2. Western blot analysis of Omp28 protein. M: Prestained protein molecular weight marker (SMO441), Lane 1: Purified protein.

Score	Expect	Identities	Gaps	Strand
593 bits(321)	2e-174	327/330(99%)	0/330(0%)	Plus/Plus

```
Query  1    ATGAATAAATTCTCCCTTGCTACAGCAGGTACTATCGTGGCAGCGCTGGTAACCAGTGTT  60
            ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  1    ATGAATAAATTCTCCCTTGCTACAGCAGGTATTATCGTGGCAGCGCTGGTAACCAGTGTT  60

Query  61   AGCGTGAATGCGGCAACAGATACTACTAAAACAAACGTTACGCCTAAAGGTATGAGCTGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AGCGTGAATGCGGCAACAGATACTACTAAAACAAACGTTACGCCTAAAGGTATGAGCTGC  120

Query  121  CAGGAGTTTGTTGACCTCAATCCGCAGACGATGGCGCCAGTCGCTTTCTGGGTGCTGAAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CAGGAGTTTGTTGACCTCAATCCGCAGACGATGGCGCCAGTCGCTTTCTGGGTGCTGAAT  180

Query  181  GAAGATGAAGATTTTAAAGGCGGGGACTACGTAGATTTCCAGGAAACTGAGGCGACAGCA  240
            |||||||||||||||||||||||||||||| |||||||||||||||||| ||||||||
Sbjct  181  GAAGATGAAGATTTTAAAGGCGGGGACTACGTTGATTTCCAGGAAACTGAGACGACAGCA  240

Query  241  GTGCCGCTAGCCGTTGAGCTTTGTAAGAAAAACCCGCAGAGTGAATTAAGCAAAATAAAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GTGCCGCTAGCCGTTGAGCTTTGTAAGAAAAACCCGCAGAGTGAATTAAGCAAAATAAAA  300

Query  301  GACGAAATCAAGAAAGAACTCTCAAAATAA  330
            ||||||||||||||||||||||||||||||
Sbjct  301  GACGAAATCAAGAAAGAACTCTCAAAATAA  330
```

Fig. 3. Sequence Alignment for *Omp28* gene from *Salmonella* Typhi and Typhimurium. Nucleotide sequences showed 99% homology between *S.* Typhi and Typhimurium in NCBI blast.

In earlier reports Omp28 was also reported to be an immunogenic protein. NEVES-FERREIRA et al. (2004) first characterized Omp28 protein in *S.* Typhi, and on the basis of its sero-reactivity reported it as an immunogenic protein. SAXENA et al. (2014) cloned,

expressed the protein in an *E. coli* host system and tested its immune-potential in a mouse model and observed that the mice produced a strong humoral response. On challenging it with a virulent strain of *S.* Typhi, the mortality was also significantly reduced. This study further confirms the immune-potential of Omp28 in the genus *Salmonella*. Upon comparison of Omp28 sequences between *S.* Typhi and Typhimurium, 99% of homology was observed (Fig. 3). Therefore, on this basis, the presumption may be made that the structures of both proteins may be very similar, and there is a strong possibility that Omp28 from *S.* Typhimurium is an immunogenic protein.

In conclusion, the findings of this study, along with earlier reports, indicate that Omp28 is an antigenic protein. So, it is suggested that further animal protection studies should be carried out using the Omp28 protein of *S.* Typhimurium, because this protein may offer an alternative to existing vaccines for controlling salmonellosis, and could avoid the immense expense of treatment. Thus it may be used as a candidate for development of a recombinant subunit vaccine against non typhoidal salmonellosis in the future.

Acknowledgements
The authors are thankful to the Dean of the College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture & Technology, Pantnagar for providing necessary facilities for this study.

Conflicts of interest
The authors declare that they have no conflict of interest.

References

BHAT, N. H., S. K. JAIN (2010): Immunogenic evaluation of a recombinant 49-kilodalton outer membrane protein of Salmonella typhi as a candidate for a subunit vaccine against typhoid. J. Infect. Dis. Immun. 2, 30-40.

BHATNAGAR, N., W. MULLER, S. SCHLECHT (1982): Proteins from Salmonella R-mutants mediating protection against Salmonella typhimurium infection in mice I. Preparation of proteins free from lipopolysaccharide using various chromatographic methods. Zentralbl. Bakteriol. Mikrobiol. Hyg A. 253, 88-101.

CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC) (2016): National Salmonella Surveillance Annual Report, 2013. Atlanta, Georgia: US Department of Health and Human Services.

De ANDRADE, C. M., A. G. P. FERREIRA, J. D. DA SILVA, H. J. NASCIMENTO, J. G. D. S. JUNIOR (1998): Chemical and immunological characterization of a low molecular weight outer membrane protein of Salmonella typhi. Microbiol. Immunol. 42, 521-526.
DOI: 10.1111/j.1348-0421.1998.tb02319.x

FAVIER, G. I., C. S. L. ESTRADA, V. L. OTERO, M. E. ESCUDERO (2013): Prevalence, antimicrobial susceptibility, and molecular characterization by PCR and pulsed field gel electrophoresis (PFGE) of *Salmonella* spp. isolated from foods of animal origin in San Luis, Argentina. Food Control 29, 49-54.

DOI: 10.1016/j.foodcont.2012.05.056

GIL-CRUZ, C., S. BOBAT, J. L. MARSHALL, R. A. KINGSLEY, E. A. ROSS, I. R. HENDERSON, D. L. LEYTON, R. E. COUGHLAN, M. KHAN, K. T. JENSEN, C. D. BUCKLEY (2009): The porin OmpD from nontyphoidal *Salmonella* is a key target for a protective B1b cell antibody response. Proc. Natl. Acad. Sci. 106, 9803-9808.

DOI: 10.1073/pnas.0812431106

HAMID, N., S. K. JAIN (2008): Characterization of an outer membrane protein of *Salmonella enterica* serovar Typhimurium that confers protection against typhoid. Clin. Vaccine Immunol. 15, 1461-1471.

DOI: 10.1128/CVI.00093-08

HARAGA, A., M. B. OHLSON, S. I. MILLER (2008): *Salmonellae* interplay with host cells. Nature Rev. Microbiol. 6, 53-66.

DOI: 10.1038/nrmicro1788

JHA, R., A. KUMAR, A. SAXENA, M. PANDEY, R. KUMAR, M. K. SAXENA (2015): Heterogeneous expression and functional evaluation of in silico characterized recombinant OmpC of *Salmonella* Typhimurium as a functional poultry vaccine to eradicate zoonotic transmission. Afr. J. Biotechnol. 14, 2862-2870.

DOI: 10.5897/AJB2015.14865

JOHN, T. J (1996): Emerging and re-emerging bacterial pathogens in India. Indian J. Med. Res. 103, 4-18.

LAN, N. P. H., T. L. T. PHUONG, H. N. HUU, L. THUY, A. E. MATHER, S. E. PARK, F. MARKS, G. E. THWAITES, N. V. V. CHAU, C. N. THOMPSON, S. BAKER (2016): Invasive Non-typhoidal Salmonella infections in Asia: clinical observations, disease outcome and dominant serovars from an infectious disease hospital in Vietnam. PLoS Negl. Trop. Dis. 10, e0004857.

DOI: 10.1371/journal.pntd.0004857

LEE, J. S., I. D. JUNG, C. M. LEE, J. W. PARK, S. H. CHUN, S. K. JEONG, T. KWUN HA, Y. K. SHIN, D. J. KIM, Y. M. PARK (2010): Outer membrane protein of *Salmonella enterica* serovar Typhimurium activates dendritic cells and enhances Th1 polarization. BMC Microbiol. 10, 263-267.

DOI: 10.1186/1471-2180-10-263

LOPEZ, M. E., H. S. GARCIA, L. A. MALO (2012): Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. Food Res. Int. 45, 713-721.

DOI: 10.1016/j.foodres.2011.04.043

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, R. J. RANDALL (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

MANAT, Y., A. V. SHUSTOV, E. EVTEHOVA, S. Z. ESKENDIROVA (2016): Expression, purification and immunochemical characterization of recombinant OMP28 protein of Brucella species. Open Vet. J. 6, 71-77.

DOI: 10.4314/ovj.v6i2.1

MURUNGKAR, H. V., H. RAHMAN, A. KUMAR, D. BHATTACHARYA (2005): Isolation, phage typing and antibiogram of *Salmonella* from man and animals in northeastern India. Indian J. Med. Res. 122, 237-242.

NEVES-FERREIRA, G. C., C. M. ANDRADE, M. A. V. SANTOS, J. PERALES, H. J. NASCIMENTO, J. G. DA SILVA Jr. (2004): Complete amino acid sequence and location of Omp28, an important immunogenic protein from *Salmonella enterica* serovar Typhi. Protein J. 23, 71-77.

DOI: 10.1023/B:JOPC.0000016260.03793.30

NEWELL, D. G., M. KOOPMANS, L. VERHOEF, E. DUIZER, A. AIDARA-KANE, H. SPRONG (2010): Food-borne diseases - The challenges of 20 years ago still persist while new ones continue to emerge. Int. J. Food Microbiol. 139, 3-15.

DOI: 10.1016/j.ijfoodmicro.2010.01.021

PANDEY, M., M. K. SAXENA (2015): Cloning and immunopotential analysis of Omp 28 of *Salmonella* Typhimurium for the development of subunit vaccine for poultry salmonellosis. Indian J. Poult. Sci. 50, 138-142.

RAMYA, P., T. MADHAVARAO, L.V. RAO (2012): Study on the incidence of *Salmonella enteritidis* in poultry and meat samples by cultural and PCR methods. Vet. World 5, 541-545.

DOI: 10.5455/vetworld.2012.541-545

SAMBROOK, J., D. W. RUSSEL (2001): Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press.

SAXENA, A., M. PANDEY, R. JHA, M. K. SAXENA (2014): Immunogenic evaluation of recombinant outer membrane protein of *Salmonella* Typhi in mice to develop subunit vaccine against typhoid. In: Proceedings of International Seminar on New Frontiers in Biotechnology Functional Genomics and Proteomics, Bareilly, Uttar Pradesh, India, p.5.

SAXENA, A., S. TAMULY, M. K. SAXENA (2012): Cloning, sequencing and In silico characterization of Omp 28 of *Salmonella* Typhi (strain MTCC 733) to develop r-DNA vaccine for typhoid fever. J. Nat. Sci. Biol. Med. 3, 133-138.

DOI: 10.4103/0976-9668.101885

TOWBIN, U., T. STAEHELIN, J. GORDON (1979): Electophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. 76, 4350-4354.

DOI: 10.1073/pnas.76.9.4350

VERMA, J. C., V. P. SINGH, B. R. SINGH, B. R. GUPTA (2001): Occurrence of *Salmonella* serotypes in animals in India - VII. J. Infect. Dis. 22, 51-55.

WILSON, K (1987): Preparation of genomic DNA from bacteria. In: Current Protocols in Molecular Biology (Ausabel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, K. Struhl, Eds.) John Wiley & Sons, New York, 2. 4. 1-2. 4. 5.

YOUSEFI, S., M. TAHMOORESPUR, M. H. SEKHAVATI (2016): Cloning, expression and molecular analysis of Iranian *Brucella melitensis Omp25* gene for designing a subunit vaccine. Res. Pharm. Sci. 11, 412-418.

DOI: 10.4103/1735-5362.192493

Received: 26 May 2017

Accepted: 14 December 2017

PANDEY, M., M. K. SAXENA, A. SAXENA, R. JHA, S. K. RASTOGI, R. KUMAR: Kloniranje, izražajnost i pročišćavanje bjelančevine 28 iz vanjske membrane *Salmonelle enterice* serovar Typhimurium za razvoj podjediničnog cjepiva - kratko priopćenje. Vet. arhiv 88, 559-568, 2018.

SAŽETAK

Salmonella Typhimurium glavni je gastrointestinalni patogen koji je globalna prijetnja ljudskome zdravlju. Javnozdravstveni problemi povezani s ovim organizmom povećali su se do te mjere da je postao glavno pitanje na koje se traže brojni odgovori. Bakterija postaje otporna na najčešće dostupne antibiotike, a ograničavajuća uporaba cjepiva povezana je s kratkotrajnim imunitetom. Zbog toga postoji hitna potreba za razvoj učinkovitog cjepiva. Vanjske bjelančevine membrane (engl. Outer membrane proteins, Omps) salmonele dokazale su svoju sposobnost kandidata za razvoj cjepiva koje bi se koristilo u prevenciji salmoneloze. S tim je ciljem u ovom radu, pod uvjetima IPTG indukcijskog sustava, provedeno umnažanje i kloniranje te provjerena izražajnost gena za Omp28 iz *Salmonelle* Typhimurium. Tako dobivena rekombinantna bjelančevina pročišćena je i testirana s obzirom na antigenu sposobnost. Antigena sposobnost pročišćene bjelančevine potvrđena je uporabom Western blot metode s antiserumom protiv Omps-a iz *S.* Typhimurium dobivenim od zeca. Genom *Omp28* umnožen je kao 330bp produkt. Bjelančevina je imala približno 28 kDa i Western blot analizom pokazala je izraženost jakog signala. Ovim je istraživanjem zaključeno da Omp28 može poslužiti kao učinkoviti kandidat za razvoj r-DNA cjepiva protiv salmoneloze.

Ključne riječi: kloniranje; izražajnost; Omp28; *Salmonella* Typhimurium; cjepivo; Western blot