



Comparison of recombinant and whole cell leptospiral antigen against different cattle serovars of leptospira

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Received: 6 January 2015; Accepted: 8 March 2015

ABSTRACT

The outer membrane protein, viz. OmpL1 of *Leptospira interrogans Icterohaemorrhagiae* was cloned into pET15b vector. The recombinant colonies were expressed in *E. coli* BL21 DE3 cells. The expressed proteins were checked in SDS-PAGE and western blot analysis. Similarly, the whole cell antigen was prepared using *Leptospira interrogans Icterohaemorrhagiae* culture. The cross reactivity of recombinant and whole cell leptospiral antigen against 5 different cattle serovars viz., Hebdomadis, Australis, Hardjo, Pomona, and Ballum were tested using ELISA. All the five cattle serovars gave statistically significant values for cross reaction against recombinant antigen in comparison to whole cell antigen. This clearly indicated that in comparison to whole cell antigen OmpL1 an outer membrane protein cross reacted with different cattle serovars and could be used as an effective diagnostic antigen for leptospirosis.

Key words: Cross reactivity, Enzyme linked immunosorbent assay, Recombinant leptospiral outer membrane protein, Whole cell leptospiral antigen

Leptospirosis is considered to be the most widespread zoonotic disease in the world. Reservoir host with chronic tubular infection transmit pathogenic *Leptospira* species to new host through urinary shedding (Farr 1995). The disease occurs widely in developing countries such as Brazil and India and is reemerging in developed countries. Leptospiral infection can range in severity from an unapparent infection to death from renal or hepatic failure (Feigin and Anderson 1975).

Currently used whole cell vaccine involves a humoral immune response to the serovars specific carbohydrate antigens of leptospiral lipopolysaccharides (LPS) (Midwinter *et al.* 1990). Currently available leptospiral vaccine has low efficacy, produce only short term immunity and local reactions (Bolin *et al.* 1991).

The whole cell leptospira vaccine containing lipopolysaccharide (LPS) conferred protective immunity against challenge with homologous leptospira alone (Sonrier *et al.* 2000). Unlike leptospiral LPS, the outer membrane proteins viz. OmpL1 and LipL41 are surface exposed and antigenically conserved among pathogenic *Leptospira* species. Since, highly conserved protein is needed for effective diagnosis of *Leptospira* against different serovars, the study was focused to compare the cross-reactivity of recombinant antigen and whole cell

antigen against different cattle serovars of *Leptospira* in order to understand the cross reacting potential of outer membrane protein, viz. OmpL1 to be used as a effective antigen in diagnosis of leptospirosis in cattle.

MATERIALS AND METHODS

Cultivation of Leptospira and collection of sera samples: *L. icterohaemorrhagiae* reference strain available in the Department of Biotechnology, Madras Veterinary College, Chennai, was inoculated aseptically in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium and the growth was observed in dark field microscope to check for the presence of the organism. Hyper-immune serum raised against *L. icterohaemorrhagiae* in rabbits was used in this study.

Polymerase chain reaction (PCR) and sequencing of OmpL1 gene: DNA was extracted from leptospira culture using high salt method described by Lahiri *et al.* (1991). The published primers and temperature profile for the amplification of OmpL1 gene were used as described by Haake *et al.* (1991).

Nde I

Forward Primer

5' AAGGAGAAGCATATGATCCGTAACATAAGT 3'

Reverse Primer

*Bam*HI

5' TTGATTGGATCCTTAGAGTTCGTGTTTATA 3'

The amplified product (5µl) was checked in 1.5% agarose gel electrophoresis along with 1Kb DNA molecular

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weight marker. The OmpL1 gene product was purified using Qiagen PCR product purification kit and sequenced. The nucleotide sequence of OmpL1 gene was compared with other *Leptospira* sequences using BLAST analysis.

Cloning and expression of OmpL1 gene in prokaryotic vector: The PCR amplicon and the expression vector (pET15b) were digested with NdeI and BamHI restriction enzymes and ligated using T4 DNA ligase in the ratio 3:1. The ligation was carried out at 16°C overnight. The 3 µl of the ligated mixture was transformed into *E. coli* BL21 DE3 cells. The transformants were spread onto LB ampicillin plates for selection of transformed colonies.

The colonies were screened for colony PCR and plasmid was isolated from colonies positive by PCR. The plasmids were then digested with NdeI and BamHI enzymes for checking the insert release in 1.5% agarose gel electrophoresis.

The positive bacterial cultures were induced with 1mM IPTG and similarly, the positive culture without IPTG was used as uninduced control. The induced and uninduced cells were lysed by heat denaturation and resolved in 12% SDS-PAGE alongwith protein molecular weight marker.

The recombinant OmpL1 fusion proteins were purified by affinity chromatography using Ni²⁺-NTA affinity column as per the invitrogen protocol. The 5th h and 6th h induced lysed cells were transferred to nitrocellulose membrane (NCM) from PAGE. The NCM was treated with anti-rabbit polyclonal serum raised against *Leptospira* to check the specificity of the expressed protein along with pre-stained protein marker by western blot analysis.

Preparation of whole cell antigen: The 7 days old (10⁸) *Leptospira* culture was pelleted by centrifugation and it was suspended in 1ml of phosphate buffer saline and heat inactivated at 55°C for 30 min and used as whole cell antigen (Bey and Johnson 1982).

Cattle sera samples: Sera samples were collected from suspected cattle from different regions of Tamil Nadu, India and screened for leptospirosis using micro agglutination test as per Smythe (2002).

Enzyme linked immunosorbent assay using recombinant antigen: The ELISA was performed as per Cullen *et al.* (2002). Fifty microgram concentration of recombinant antigen (OmpL1) and whole cell antigen were coated in ELISA plate. A total of 210 sera samples from different serovars, viz. Hebdomadis, Australis, Hardjo, Pomona, and Ballum were checked for cross reactivity. The OD values were measured at 492nm using ELISA reader. The relative sensitivity and specificity of ELISA for detection of leptospiral antibodies using recombinant antigen was determined in comparison with MAT as described by Senthilkumar *et al.* (2008).

RESULTS AND DISCUSSION

The expected amplicon size of 963bp for OmpL1 gene was observed in 1.5% agarose gel. BLAST analysis revealed that the OmpL1 nucleotide sequence (GQ463696.1) showed 99% homology with other *Leptospira* sequences available

in gennank. After cloning, the recombinant colonies were analyzed by colony PCR (Fig. 1) and insert release by restriction enzyme digestion (Fig. 2).

The IPTG induced and uninduced cells were lysed by

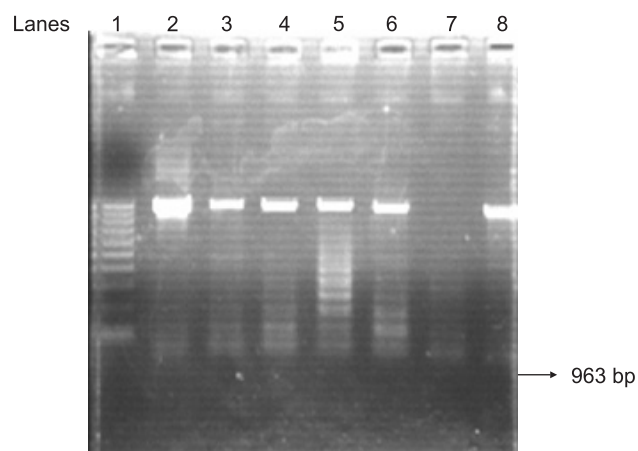


Fig. 1. Colony PCR for screening of the recombinant OmpL1 gene.

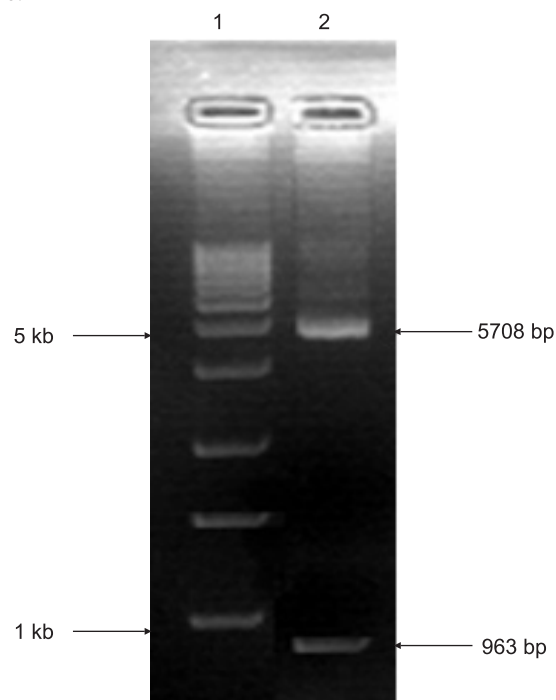


Fig. 2. Demonstration of insert DNA release by restriction digestion of recombinant plasmid OmpL1 gene.

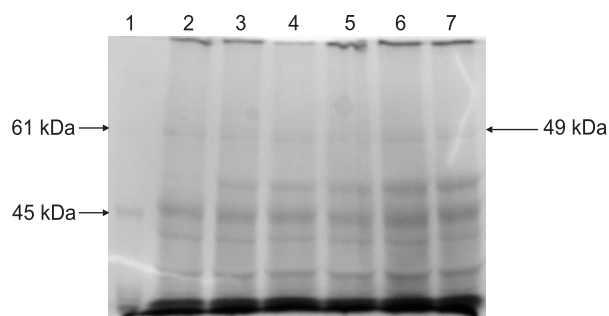


Fig. 3. SDS - PAGE analysis of OmpL1 gene expressed protein.

Table 1. Student unpaired T- test of two samples assuming equal variance

Serovars	Antigen		t Stat value	P (T<=t) one-tail
	Whole cell serum samples (n)	Recombinant serum samples (n)		
Hebdomadis	48	48	4.10172	4.36E-05*
Australis	45	45	6.84392	4.94E-10*
Hardjo	36	36	7.78457	2.28E-11*
Pomona	36	36	9.47221	1.81E-14**
Ballum	45	45	12.0935	1.04E-20**

N, Number of serum samples; *, significant ($P < 0.05$); **, highly significant ($P < 0.01$).

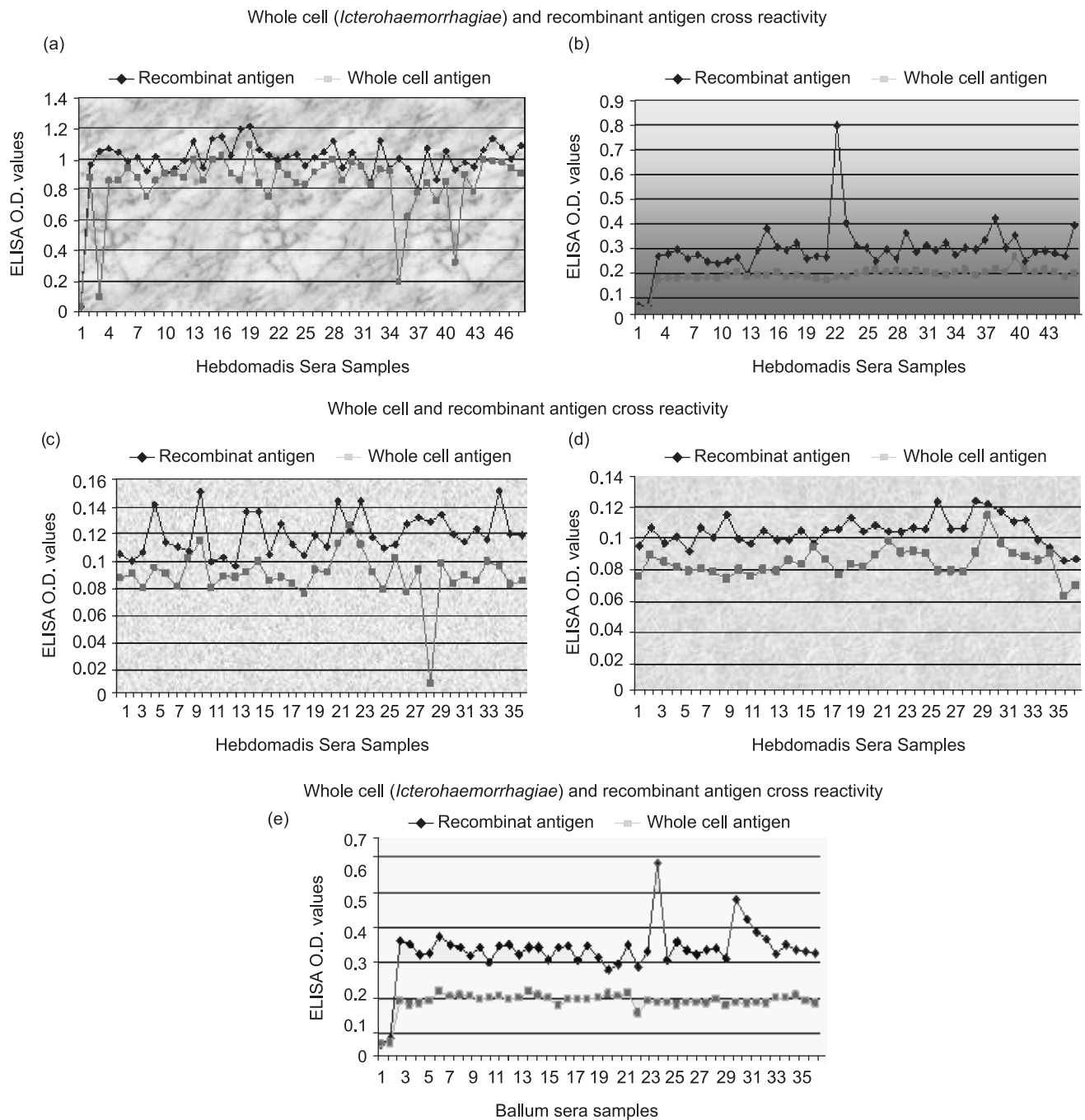


Fig. 4. (a-e) Cross reactivity of recombinant and whole cell antigen. a. Hebdomadis serovar; b. Australis serovar; c. Hardjo serovar; d. Pomona serovar; e. Ballum serovar.

heat denaturation and resolved in 12% SDS-PAGE. The expressed recombinant OmpL1 protein was noticed only in the induced culture at different hours of induction with the molecular weight of 49kDa (Fig. 3) .

The nickel (Ni²⁺-NTA) affinity column purified protein was checked in 12% SDS-PAGE and a band with mol wt of 49kDa was observed. The specificity of recombinant purified protein was checked with *Leptospira* antiserum raised in rabbits. The antiserum reacted with protein band of molecular weight of 49 KDa. The cross reactivity of recombinant and whole cell antigen against different serovars were presented in Figs 4a-e. All the sera samples reacted with recombinant antigen gave high O.D values in comparison to whole cell antigen. The unpaired T-test was used to determine the significance of cross reactivity between whole cell antigen and the recombinant antigen and the results were interpreted in Table 1.

Although, leptospiral bacterins may protect animals from developing clinical signs of the disease, they are ineffective in preventing leptospiroemia and renal shedding (Levett 2001). The immunity developed by whole cell vaccine has been demonstrated. However, cross protection against many of the 250 different serovars of pathogenic *Leptospira* is lacking, as because whole cell vaccine which contain lipopolysaccharides conferred protective immunity against challenge with homologous serovar but not against the heterologous leptospira (Sonrier *et al.* 2000). The number of outer membrane proteins such as OmpL1 and LipL32, LipL36, LipL41 and LigA and LigB has been cloned and characterized (Andre-fontaine *et al.* 2003). Nally *et al.* (2001) reported that OmpL1 is a surface exposed protein and also expressed during infection and therefore, in this study this outer membrane protein was cloned and expressed. The cross- reaction of OmpL1 was verified by ELISA using recombinant OmpL1 protein as coated antigen in serum samples from 385 leptospira patients and cross reactivity was demonstrated (Cullen *et al.* 2002). In this study, cross reaction against whole cell antigen and recombinant antigen were verified against 5 different cattle serovars using ELISA. In comparison to MAT assay, the recombinant OmpL1 antigen showed 98.7% sensitivity and 95% specificity. In statistical analysis, Dong *et al.* (2008) used Kappa value to determine the cross reactivity between serovars using qualitative data whereas in this study, the unpaired tests was used to determine the cross reactivity among the different serovars using quantitative data. All the five cattle serovars tested gave statistically significant results for cross reaction against recombinant outer membrane proteins in comparison to whole cell antigen. It clearly indicated that in comparison to whole cell antigen the outer membrane protein reacted with other heterologous serovars of leptospira under *in-vitro* condition and therefore,

it could be used as a potential antigen for diagnosis of leptospirosis.

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