Cloning and sequencing of 28 kDa outer membrane protein gene of *Brucella melitensis* Rev. 1

Pallab Chaudhuri *, S Vinod Kumar, Rajeev Prasad, S K Srivastava¹ & MP Yadav
National Biotechnology Centre, Indian Veterinary Research Institute, Izatnagar 243 122, India

Received 6 April, 2005

*Brucella melitensis* is an organism of paramount zoonotic importance. The 28 kDa outer membrane protein (OMP) is one of the immunodominant antigens of *B. melitensis*. The gene encoding 28 kDa OMP (omp28) has been amplified from *B. melitensis* Rev. 1 strain. A PCR product of 753 bp, encoding complete omp28 gene of *B. melitensis*, was obtained. The gene was further cloned and sequenced. The nucleotide sequence of *B. melitensis* Rev. 1 strain showed substitution of 2 nucleotides from that of 16M strain.

**Keywords:** *Brucella melitensis*, Cloning, Outer membrane protein

Brucellosis is a major zoonotic disease widely distributed in both animals and humans, especially in developing countries¹. *Brucella* are gram negative, facultative intracellular bacteria which causes abortion and infertility in cattle, sheep and goats and undulant fever, endocarditis and osteomyelitis in humans².³. Vaccination against animal brucellosis is commonly performed by using live attenuated *Brucella* strains. These vaccines have certain disadvantages like its incomplete avirulence and interference in diagnosis of clinical infection. The identification of *Brucella* antigens with the capacity to elicit protective immune response is of great interest for the development of sub-cellular vaccines that do not have the drawback of live attenuated vaccine. *Brucella melitensis* Group 3 outer membrane proteins (OMP) have been identified as immunodominant antigens⁴.⁵. The 28 kDa OMP of *B. melitensis* belongs to group 3 antigens. In the present report, we have described the amplification and cloning of 28 kDa OMP from *B. melitensis* Rev. 1 strain. Rev. 1 is a smooth attenuated strain of *B. melitensis* and is used for vaccination of sheep and goats against *B. melitensis* infection.

**Bacterial strains and plasmid** — The vaccine strain, *Brucella melitensis* Rev. 1 was used in the present study to clone 28 kDa OMP gene. The organism was grown in tryptic soya agar for extraction of nucleic acid. *E. coli* JM109 was used as host and plasmid pTZ57R (MBI Fermentas, Canada) was used as cloning vector.

**Detection of 28 kDa OMP gene** — *Brucella melitensis* DNA encoding 28 kDa outer membrane protein gene was amplified by PCR. Primers were designed based on available DNA sequence (U30815). DNA amplification was carried out in 50 µl of reaction mixture containing 10 mM, Tris.Cl (pH 8.3); 1.5 mM, MgCl₂; 80 mM, KCl; 0.5 µM of each oligonucleotide primers; 200 µM, dNTPs; 50 ng of genomic DNA and 2 units of Taq DNA polymerase. Thirty cycles of thermal reaction were carried out with the following conditions — denaturation at 90°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 1 min. Amplified product was confirmed by nested PCR.

**Cloning of 28kDa OMP gene** — PCR amplified product was eluted from agarose gel using Qiaex II gel extraction kit (Qiagen Inc, USA). Eluted DNA fragment was ligated to pTZ57R vector (Inst/TA clonig Kit, MBI Fermentas) at 16°C for 8 hr. The newly generated construct was designated as pOMP28. The recombinant construct was transformed to *E. coli* JM109 competent cells. Transformants were plated on LB agar supplemented with X-gal/ IPTG and ampicillin. Recombinant clones were screened based on blue/white colour colony selection.

**Sequencing of omp28 gene** — The cloned omp28 gene was sequenced by dideoxy chain termination method. The nucleotide sequence has been submitted to GenBank. Nucleotide and deduced amino acid sequences of omp28 gene were analyzed by using ‘EditSeq’ program of Lasergene software (DNASTAR, Madison, USA).

The 28-kDa outer membrane protein of *Brucella* belongs to group 3 antigen. In the present study we

---

* Corresponding author -
E-mail: pallab@ivri. up.nic.in

¹ Division of Bacteriology & Mycology, IVRI,
Izatnagar 243 122, India
amplified and cloned omp28 gene of *B. melitensis* Rev. 1 strain. Rev1 strain is used for vaccination of sheep and goats against *B. melitensis* infection. We also compared the sequence homology of omp28 gene of *B. melitensis* Rev. 1 strain and *B. melitensis* 16M strain. The amplification of 28 kDa outer membrane protein gene from *Brucella melitensis* Rev. 1 strain was achieved by polymerase chain reaction using a set of primers which were designed based on the sequence with accession no U30815 (Luther et al., 1996). On amplification, a desired product of 753 bp DNA fragment was observed on 1.5% agarose gel (Fig. 1). Amplification of a 553 bp nested PCR product confirmed the specificity of the amplified gene (Fig. 1).

The omp28 gene was further analyzed by nucleotide sequencing. The PCR product encoding 28 kDa OMP gene was eluted from agarose gel and ligated to pTZ57R vector (Inv/TA cloning kit, MBI Fermentas, Canada). The resulting construct was designated as pOMP28. Upon transformation of pOMP28 into *E. coli* JM109 competent cells, a large number of recombinant clones were obtained which produced while colour colonies on LB plate supplemented with X-gal and IPTG. Presence of correct insert in the recombinant clone confirmed by the release of 753 bp insert by digestion of the isolated plasmid with BamHI and HindIII (Fig. 2). Restriction analysis of the recombinant clone with NheI and PstI also yielded correct restriction pattern (data not shown).

The recombinant clone pOMP28 was used to sequence the omp28 gene insert. The nucleotide sequence of omp28 gene of *B. melitensis* Rev. 1 has been submitted to GenBank and assigned the accession number AY634231. The nucleotide sequence of omp28 gene contained a 753 bp open reading frame (Fig. 3) which codes for 250 amino acids. The deduced amino acid sequence of omp28 has the estimated molecular mass of 26537 Daltons.

---

**Fig. 1** — PCR amplification of omp28 gene. [Lane M. 100 bp DNA ladder; Lane 1. 753 bp PCR amplified omp28 gene; Lane 2. 553 bp nested PCR product]

**Fig. 2** — Release of cloned insert (omp28 gene) from pOMP28. [Lane M. 100 bp DNA ladder, Lane 1. pOMP28 plasmid digested with BamHI and HindIII releasing 753 bp omp28 gene insert from 2.8 kb pTZ57R plasmid. Lane 2. 753 bp PCR amplified omp28 gene]

**Fig. 3** — Nucleotide sequence of omp28 gene (Open reading frame 1-753) of *B. melitensis* Rev. 1
The primary structure of the protein, analyzed by the software, showed presence of 24 strongly basic amino acids, 24 strongly acidic amino acids and 96 hydrophobic amino acids. There are two nucleotide substitutions at position 327 and 664 in the Rev. 1 strain of *B. melitensis* from that of 16M strain. The nucleotide sequence variation resulted in change of one amino acid (valine to leucin) at position 222 in Rev. 1 strain. *Brucella* group antigens are described according to their molecular weight. Group 3 antigens were designated to possess molecular mass of approximately 30 kDa and they are unchanged by heating of samples before electrophoresis. Lindler *et al.* have reported that 28 kDa outer membrane protein of *B. melitensis* 16M is a member of group 3 antigen which showed immunoreactivity with human convalescent sera from patients infected with *Brucella* species. It has been suggested that Omp28 may be a good candidate for vaccine and diagnostic purposes. Presence of similar protein in the vaccine strain of *B. melitensis* might have a particular utility for developing a subcellular vaccine and a diagnostic reagent.

**References**