

Soluble Expression of OmpA from *Haemophilus parasuis* in *Escherichia coli* and Its Protective Effects in the Mouse Model of Infection

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***Haemophilus parasuis* causes contagious porcine Glässer's disease leading to severe losses in the swine industry. In this study, we established an efficient *Escherichia coli*-based system for the expression of *H. parasuis* major outer-membrane protein (MOMP) that has been known as a good vaccine candidate against Glässer's disease. Use of an *E. coli*-derived pelB leader sequence made it possible to produce recombinant MOMP (rMOMP) as the soluble forms without an additional refolding process. Using two different animal models, it was evaluated that the rMOMP was capable of inducing a significant immune response and providing protection against *H. parasuis* infection.**

Keywords: *Haemophilus parasuis*, soluble expression, pelB leader sequence, antigen, OmpA, *Escherichia coli*

Gram-negative rod-type *Haemophilus parasuis* is an etiological agent that causes Glässer's disease, a condition characterized by fibrinous polyserositis, polyarthritis, and meningitis. This disease leads to large losses in swine populations worldwide [1, 6]. To date, 15 serotypes of this pathogen have been identified, with serotypes 4 and 5 being prevalent among field isolates in many countries [4]. Current bacterin-type vaccines against *H. parasuis*-mediated disease provide protection only against challenge with a homologous serotype but not with a heterologous serotype [2, 5, 7]. Therefore, screening for antigenic proteins at the molecular level is needed for cross-protection.

Outer membrane proteins (OMPs) are considered to be important antigens for formulating vaccines against Gram-negative bacteria [3, 10]. *H. parasuis* OMPs also react with antibodies detected in the sera of vaccinated pigs [5,

13]. Tadjine *et al.* (2004) [8] reported that all 15 serotypes of *H. parasuis* react with monoclonal antibodies against one major OMP (MOMP) related to the OmpA family of Gram-negative bacteria, suggesting that this protein may be a good candidate for an effective diagnostic marker. Recently, the open reading frame (ORF) of this MOMP was cloned and its amino acids were found to exhibit high variation among serovars [9, 11], but recombinant MOMP (rMOMP) cross-reacted with antiserum against all the 15 serovars [9] and induced protective response in mice vaccinated against *H. parasuis* [12]. These observations suggested that rMOMP may be a good vaccine candidate against Glässer's disease.

Recombinant protein production is a well-established technology and an efficient strategy for the cost-effective preparation of a protective antigen for the development of vaccines against pathogenic organisms. However, it was observed that rMOMP by *E. coli* was expressed as only the insoluble forms that subsequently require an additional refolding process, which increases production costs [11, 12]. The present study describes the soluble MOMP expression in *E. coli* through its periplasmic location. Furthermore, protective immunity of soluble rMOMP against *H. parasuis* infection was evaluated in the vaccinated mouse model.

For the expression of rMOMP in the periplasm of *E. coli*, we examined use of the native MOMP and *E. coli*-derived pelB leader sequence (LS). The gene encoding full-length MOMP with its native LS was amplified by PCR from the genomic DNA of serotype 4 isolated in South Korea by PCR and the following primers: forward primer for the full-length gene, 5'-GTACATATGAAAAA TCTTTAATTGCATTAG-3', forward primer for the mature gene without the signal sequence, 5'-GTACATATGGCT CCACAAGCTAACACTT-3', and reverse primer 5'-ATA CTCCGAGCATAGAACTTCTTTTGAAC-3' (underlined nucleotides indicate the sites for the restriction enzymes of *NdeI* and *XhoI*, respectively). The full-length MOMP gene and the mature gene with a pelB LS adjacent to the N-

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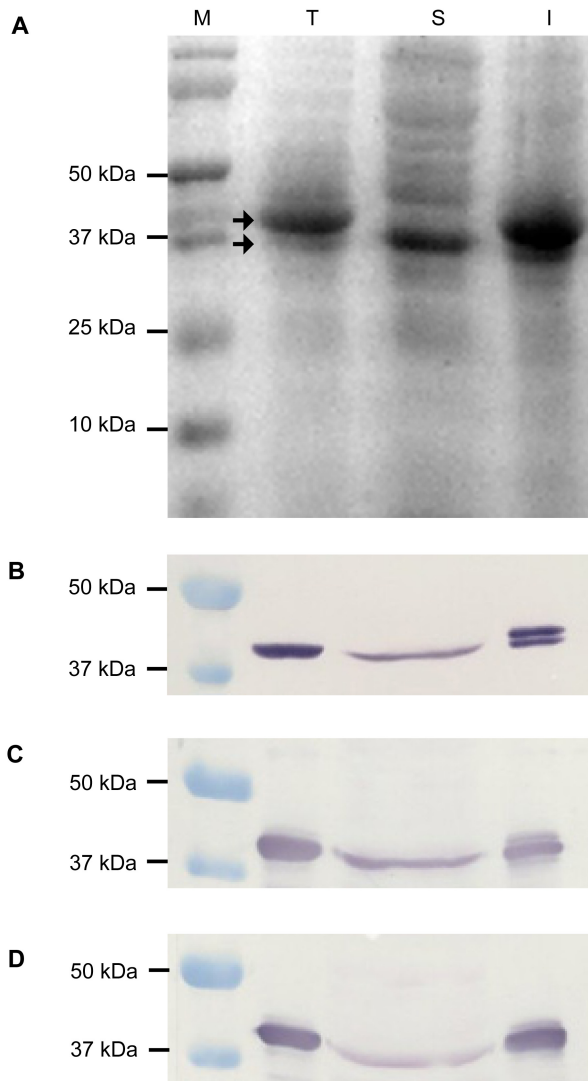


Fig. 1. SDS-PAGE (A) and Western blot analyses of *H. parasuis* OmpA protein expressed in *E. coli* BL21(DE3)/pKPM-pelB-mMOMP using an anti-histidine antibody (B), and the antiserum collected from mice inoculated with whole *H. parasuis* serotype 4 (C) and 5 (D) cells.

After IPTG induction of MOMP expression, total and soluble fractions from *E. coli* BL21(DE3)/pET28-pelB-mMOMP were separated by 12% SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was then incubated with an anti-histidine antibody or appropriate antiserum. Lane M: standard molecular weight marker; T: total cell lysates; S: soluble fraction; I: insoluble fraction. The arrows indicate the OmpA expressed in *E. coli*.

terminal were inserted into the *NdeI/XhoI*-digested pEKPM where the DNA region between *BglIII/NdeI* in pET-28b(+) was replaced by the region between the same enzyme sites in pET-22b(+); these were designated as pEKPM-MOMP and pEKPM-pelB-mMOMP, respectively. In the constructs, the MOMP genes were placed under the control of a T7 promoter and fused to a poly-histidine at their C-termini.

E. coli BL21(DE3)/pEKPM-MOMP did not result in any shift of rMOMP expression from insoluble to soluble,

Table 1. Titers of sera-specific antibodies in guinea pigs.

Group	Antibody titers ^a (OD ₄₉₂ of sera diluted 400-fold)
pEKPM-pelB-mMOMP	1.429
pEKPM	0.072
Non-immunized group	0.054

^aSpecific pathogen-free (SPF) guinea pigs were intraperitoneally immunized with the trial vaccine containing a soluble protein extract (250 µg) from *E. coli* BL21(DE3)/pEKPM-pelB-mMOMP or the blank control containing soluble extract (100 µg) from *E. coli* BL21(DE3)/pEKPM. A second booster was given 2 weeks after the first vaccine administration. The titers of sera from immunized mice were measured using an ELISA with rMOMP purified by Ni-NTA agarose (Qiagen, Germany). All data represent the averages from five serum samples from immunized mice.

indicating that the native signal peptide was not appropriate for expression in *E. coli* (data not shown). In *E. coli* BL21(DE3)/pEKPM-pelB-mMOMP, soluble MOMP was successfully expressed at the same size as the mature form when the pelB signal peptide was cut with signal peptidase (Fig. 1A and 1B). The antigenicity of the soluble rMOMP was evaluated by Western blot analysis with mouse-derived antiserum. The result showed that antisera against whole *H. parasuis* serotype 4 and 5 cells reacted with the soluble MOMP (Fig. 1C and 1D).

In order to further examine whether the rMOMP could induce cellular immunity, its effects on immunological responses and the ability to provide protective immunity were evaluated in a guinea pig and mouse model, respectively. For the animal experiments, crude extracts from *E. coli* expressing soluble rMOMP were used and, therefore, crude extracts from *E. coli* harboring the pEKPM vector were used as a blank control. As shown in Table 1, an ELISA antibody assay showed that levels of antibodies against rMOMP in the serum of the rMOMP-immunized group were higher than that of the vector control-immunized and unimmunized groups. These results indicated that the soluble rMOMP elicited a significant immunological response in the guinea pig model. In the mice challenged with *H. parasuis* infection, all animals in the non-vaccinated and blank control groups died within 1 day after the challenge, whereas the survival rate in the soluble rMOMP-vaccinated groups was 50% (Fig. 2). These results indicated that the soluble rMOMP offered effective protection against *H. parasuis* infection in the mouse model.

In conclusion, we established a soluble rMOMP expression system using the pelB signal sequence. Using our expression system, it was possible to produce soluble rMOMP that did not require an additional refolding process before being administered. The results from our study will be helpful for the development of *H. parasuis* vaccinations as well as the *E. coli*-based production of immunologically active and soluble OmpA proteins from Gram-negative strains.

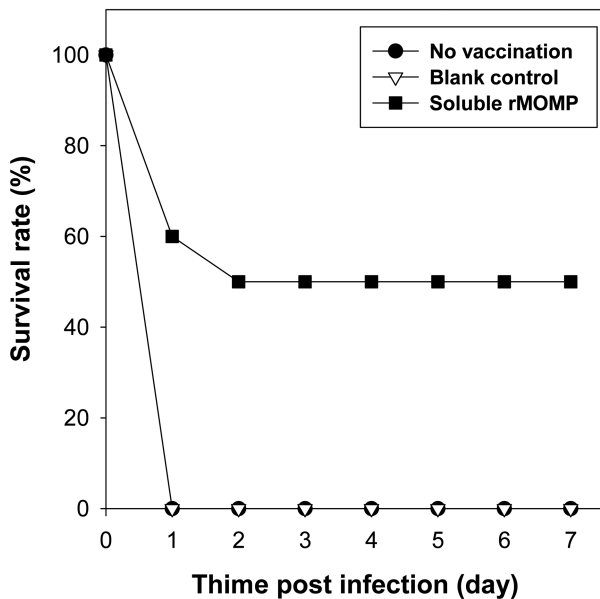


Fig. 2. Survival of mice immunized with soluble rMOMP following challenge with *H. parasuis* serotype 4.

SPF mice were intraperitoneally vaccinated with the trial vaccine containing soluble protein extract (100 µg) from *E. coli* BL21(DE3)/pEKPM-pelB-mMOMP and the blank control soluble protein extract (40 µg) from *E. coli* BL21(DE3)/pEKPM. A second booster was given 2 weeks after the first vaccine administration. All animals in the vaccinated (10 mice/group) and non-vaccinated (5 mice) groups were challenged with a lethal dose (5×10^9 CFU) of *H. parasuis* serotype 4. The survival of the challenged mice in each group was evaluated over the next 7 days.

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