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Homology analysis and cross-immunogenicity of OmpA from pathogenic *Yersinia enterocolitica*, *Yersinia pseudotuberculosis* and *Yersinia pestis*

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ABSTRACT

The outer membrane protein A (OmpA) is one of the intra-species conserved proteins with immunogenicity widely found in the family of Enterobacteriaceae. Here we first confirmed OmpA is conserved in the three pathogenic *Yersinia*: *Yersinia pestis*, *Yersinia pseudotuberculosis* and pathogenic *Yersinia enterocolitica*, with high homology at the nucleotide level and at the amino acid sequence level. The identity of *ompA* sequences for 262 *Y. pestis* strains, 134 *Y. pseudotuberculosis* strains and 219 pathogenic *Y. enterocolitica* strains are 100%, 98.8% and 97.7% similar. The main pattern of OmpA of pathogenic *Yersinia* are 86.2% and 88.8% identical at the nucleotide and amino acid sequence levels, respectively. Immunological analysis showed the immunogenicity of each OmpA and cross-immunogenicity of OmpA for pathogenic *Yersinia* where OmpA may be a vaccine candidate for *Y. pestis* and other pathogenic *Yersinia*.

1. Introduction

The genus *Yersinia* encompasses three zoonotic pathogens, *Yersinia pestis*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* (Sulakvelidze, 2000), the causative agent of a fatal disease, plague, is often transmitted by infected fleas or inspiratory aerosol. *Y. pseudotuberculosis* and *Y. enterocolitica* are entero-pathogens, the former causes mesenteric lymphadenitis and septicemia (Abe et al., 1997; Zippi et al., 2006); and the latter generate wider clinical infections among people from gastroenteritis to more invasive syndromes including mesenteric lymphadenitis and terminal ileitis (Bottone, 1997; Cover and Aber, 1989). Although there are big differences in the transmission modes and clinical manifestations among pathogenic *Yersinia,* their pathogenic processes are all characterized by translocation through the intestinal epithelium to attain residence in the Peyer's patches and their ability to defend against non-specific immunity, especially towards phagocytosis where they resist killing in macrophages and in polymorphonuclear leucocytes (PMN) (Navarro et al., 2005; Pujol and Bliska, 2005).

The outer membrane protein A (OmpA) is embedded in the outer membrane of Gram-negative bacteria. It is one of the highly conserved proteins in the family Enterobacteriaceae (Vogel and Papenfort, 2006) and therefore used as a PCR target gene for the detection of enterobacteria (Mohan Nair and Venkitanarayanan, 2006). Reports show OmpA is essential for adhesion and basolateral invasion into eukaryotes (Chandrapala et al., 2014; Kim et al., 2010); and it is related to bacterial resistance mechanisms (Llobet et al., 2009), and enhancing phagocytosis by macrophage (Sukumaran et al., 2003). In Salmonella, OmpA serves as the major immunogenic protein (Singh et al., 2003). OmpA can mediate biofilm formation for a commensal bacterium, *Sodalis glossinidius,* to colonize the tsetse fly gut (Maltz et al., 2012). We previously showed OmpA was a major immunogenic protein of pathogenic *Yersinia* (Gu et al., 2012), and it was highly homologous among different serotypes of *Y. enterocolitica* at the gene level (Li et al., 2014). Erova et al. (2013) shows the OmpA of *Y. pestis* is highly immunogenic and could protect mice against bubonic and pneumonic plague and is a possible candi-

Abbreviations: PMN, polymorphonuclear leucocytes; OmpA, the outer membrane protein A; IPTG, isopropyl-D-thiogalactopyranoside; WT, the wild-type; SPF, specific pathogen-free; ORF, the Open Reading Frame; LcrV, the low-calcium-response V antigen; McF, McFarland.

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Table 1
Strains and plasmids used for gene cloning and mutation in this study.

Strains or plasmids	Description or comments	Source or reference
Y. entercolitica strains		
Y. entercolitica Ye8629	Serogroup O:9 pYV+, isolated in Henan, China from a patient	(Gu et al., 2012)
Y. entercolitica Ye92010	Serogroup O:8 pYV+, provided by Dr H. Fukushima of the Public Health Institute of Shimane Prefecture, Japan.	(Gu et al., 2012)
Y. entercolitica 105.5R(r)	Serogroup O:9 pYV+	(Wang et al., 2011)
Y. entercolitica 105.5R(r) *ΔompA*	A deletion of *ompA* gene	This study
Y.pseudotuberculosis strains		
Y. pseudotuberculosis NX286	Isolated in Ningxia, China from a pig	This study
Y.pestis strains		
Y. pestis EV76	Pgm-	(Velan et al., 2006)
E.coli strains		
E. coli BL21(DE3)	F-ompT hsdS(rBB-mB-) galdcm(DE3)	Novagen
S17 λpir	λ-pir R6K(thi thr leu ton lacY supE recA::RP4-2Tc::Mu)	(Thoma and Schobert, 2009)
Plasmids		
pET-30a(+)	T7 promoter-based prokaryotic expression vector Kmr	Novagen
pET-30a::*ompA8629*	Expression vector with a PCR-amplified *ompA* gene in the *BamHI/SalI* site; *kmr*	This study
pET-30a::*ompA92010*	Expression vector with a PCR-amplified *ompA* gene in the *BamHI/SalI site; kmr*	This study
pET-30a::*ompA286*	Expression vector with a PCR-amplified ompA gene in the *BamHI/SalI* site; *kmr*	This study
pDS132	Conditionally replicating vector; R6K origin, mobRK4 transfer origin,sucrose-inducible sacB, Cmr	(Philippe et al., 2004)
pDS132-*ompA*	containing a fusion fragment of upstream and downstream of ompA gene	This study

date for a new-generation recombinant vaccine. Further, reports show OmpA of *Y. pestis* promotes intracellular survival and virulence in mice (Bartra et al., 2012). Pathogenic *Yersinia* are very similar genetically with more than 71% common core genomes and a 70-kb virulence plasmid (pYV) (Duan et al., 2014; Thomson et al., 2006). The V antigen is encoded on the plasmid and may be the reason a host infected with one kind of pathogenic *Yersinia* may be protected from infection by the other two (Fukushima et al., 2001; Mollaret, 1995; Roggenkamp et al., 1997). The V antigen, encoded by pVY, is not expressed stably, because it is easily lost during passages. Protein OmpA possesses cross-immunogenicity as does the V antigen. By sequence alignment, we found the *ompA* gene was highly homologous among pathogenic *Yersinia*. Using western blot, we showed cross-immunogenicity of OmpA among pathogenic *Yersinia*. Encoded on the chromosome genome, OmpA is more stable for immunogenicity and to be more of a potential vaccine candidate than the V antigen.

2. Materials and methods

2.1. Strains, plasmids and culture conditions

The strains and plasmids used for gene cloning and mutation are listed in Table 1. Bacteria were cultured using the methods reviewed by Pujol (Pujol and Bliska, 2005). The serotypes, biotypes, and pathogenicity of these strains were determined as previously described (Bottone, 1997; Thoerner et al., 2003; Wang et al., 2008). *Escherichia coli* strains were grown in Luria-Bertani medium at 37 °C. Antibiotics were used at the following concentrations: kanamycin: 50 μg/ml for agar plates, 100 μg/ml for broth; chloramphenicol, 34 μg/ml; Cefsulodin, 15 μg/ml; and Novobiocin, 2.5 μg/ml.

Identity analysis of *ompA* included 615 pathogenic *Yersinia* strains from the 1960s up to now containing 219 pathogenic *Y. enterocoltitica* strains, 262 *Y. pestis* strains and 134 *Y. pseudotuberculosis* strains. Specifically, among the 219 pathogenic *Y. enterocolitica* strains, 170 were described previously by our research group (Li et al., 2014); the total included 199 strains isolated in China from 1984 to 2014; 16 reference strains from Europe, the United States, and Japan; and four complete-genome-sequenced strains from the NCBI (Batzilla et al., 2011; Delihas, 2003; Fuchs et al., 2011; Wang et al., 2011). The bio-serotypes of *Y. enterocolitica* strains were: 133 strains 2/O:3, 3/O:3 and 4/O:3; 78 strains 2/O:9, 3/O:9 and 4/O:9; two strains 2/O:5,27; and six strains 1B/O:8 that cover the predominate bio-serotypes of pathogenic *Y. enterocolitica* in the world (Bottone, 1997; Lee et al., 1991; McNally et al., 2004; Wang et al., 2009). The 262 *Y. pestis* strains included 249 strains (either provided by the Gansu Provincial Centre for Disease Control and Prevention; Yunnan Institute of Endemic Diseases Control and Prevention; or gathered by our laboratory) isolated in China from 1962 to 2014; one reference strain EV76 (Velan et al., 2006); and 12 complete-genome-sequenced strains from the NCBI (Chain et al., 2006; Deng et al., 2002; Eppinger et al., 2010; Parkhill et al., 2001; Shen et al., 2010; Song et al., 2004; Zhang et al., 2009). The 134 *Y. pseudotuberculosis* strains included 106 strains isolated in China from 1995 to 2014; 24 reference strains (including 12 from Japan, four from France, and seven from the National Institute for the Control of Pharmaceutical and Biological Products, China (NICPBP)) and four complete-genome-sequenced strains from the NCBI (Chain et al., 2004; Eppinger et al., 2007). The sample collection and detection protocols were approved by the Ethics Review Committee from the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention. The source and host of strains are shown in Table 2.

Table 2
Source and host distribution of strains used in *ompA* identity analysis.

Source and host	*Y. pestis*	*Y. pseudotuberculosis*	*Y. enterocolitica*
Strains isolated in China	249	106	199
Diarrhea patients	26		57
Swine		17	105
Rats	14	72	19
Dogs		16	10
Marmots	134	1	
Fleas	50		
Food and environment			4
Others	25		4
Reference strains	1	24	16
Sequence strains	12[a]	4[b]	4[c]
Total	262	134	219

[a] W22703(GenBank: FR718562.1), 105.5R(GenBank: CP002246.1), Y11(GenBank: FR729477.2), 8081 (GenBank: FR729477.2, AM286415.1).
[b] A1122(GenBank: CP002956.1), Harbin35(GenBank: CP001608.1), Z176003(GenBank: CP001593.1), D182038(GenBank: CP001589.1), D106004(GenBank: CP001585.1), Angola(GenBank: CP000901.1), PestoidesF(GenBank: CP000668.1), CO92(GenBank: AL590842.1), 91,001(GenBank: AE017042.1), Antiqua(GenBank: CP000308.1), Nepal516(GenBank: CP000305.1), KIM10+(GenBank: AE009952.1).
[c] PB1/+(GenBank: CP001048.1), YPIII(GenBank: CP000950.1), IP31758(GenBank: CP000720.1), IP32953(GenBank: BX936398.1).