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Sequence diversity of the immunogenic outer membrane lipoprotein PlpE from *Mannheimia haemolytica* serotypes 1, 2, and 6

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Abstract

Mannheimia haemolytica serotype 1 (S1), S6 and S2 are the most common bacterial isolates found in shipping fever pneumonia in beef cattle. Currently used vaccines against *M. haemolytica* do not provide complete protection against the disease. Research with *M. haemolytica* outer membrane proteins (OMPs) has shown that antibodies to one particular OMP from S1, PlpE, may be important in immunity. Recombinant PlpE (rPlpE) is highly immunogenic in cattle, and the acquired immunity markedly enhanced resistance to experimental challenge. We previously demonstrated that the immunodominant epitope (R2) is located between residues 26 and 76 on the N-terminus of PlpE from a reference S1 strain (Ayalew et al., 2004). This region consists of eight hexapeptide repeats. The potential of this epitope as a vaccine or supplement to commercial vaccines is dependant on its state of conservation amongst isolates of the three serotypes. To determine this, we sequenced *plpE* genes from 32 isolates. The sequences from S1 and S6 were identical with one exception. Substantial variation was observed among sequences from S2 strains, particularly in the R2 region of the protein. These variations in S2 isolates range from 3 to 28 hexapeptide repeats. Calculated molecular weight of PlpE from S1 and S6 isolates was 37 kDa, where as PlpE from S2 strains ranged from 30 to 50 kDa. These similarities and differences were demonstrated by western blot. Competitive binding assay was used to determine that antibody against rPlpE from S1 binds native PlpE on surfaces of both S1 and S2 cells.

Keywords: Bovine respiratory disease; *Mannheimia haemolytica*; PlpE; S1; S2; S6; Epitope, Repeats

1. Introduction

Economic losses to bovine respiratory disease approach $1 billion annually in North America (Bowland and Shewen, 2000). *Mannheimia haemolytica* is the bacterium most commonly isolated from

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the lungs of shipping fever cases. *M. haemolytica* serotype 1 (S1) is responsible for approximately 60% of shipping fever caused by this gram negative bacterium, whereas S6 and S2 account for 26% and 7% of *M. haemolytica*-associated cases, respectively (Al-Ghamdi et al., 2000; Purdy et al., 1997). *M. haemolytica* is normally found in the upper respiratory tract and is inhaled into the lungs when cattle experience stress such as shipping, weaning, overcrowding, or viral infections (Frank, 1989). In controlled feedlot studies using commercial *M. haemolytica* S1 vaccines, vaccination was associated with reduced morbidity, mortality, and/or treatment costs in only 50% of the studies (Perino and Hunsaker, 1997). Therefore, an efficacious vaccine against *M. haemolytica* could significantly improve beef production.

Immunity to *M. haemolytica* is multifaceted. Antibodies against a secreted leukotoxin and against surface antigens are necessary for protective immunity to *M. haemolytica* (Shewen and Wilkie, 1988). The specific surface antigens that are important in stimulating immunity have not been determined with surety; however, we and others have presented data suggesting that outer membrane proteins (OMPs) may be important in stimulating immunity to surface antigens on *M. haemolytica* (Ayalew et al., 2004; Confer, 1993; Confer et al., 2003, 1995; Morton et al., 1995; Potter et al., 1999). Our laboratory previously identified a 45 kDa major outer membrane lipoprotein of *M. haemolytica*, PlpE (Pandher et al., 1998). Vaccination of cattle with *M. haemolytica* S1 recombinant PlpE (rPlpE) enhanced resistance against experimental challenge with *M. haemolytica* S1, and that addition of rPlpE to a commercial *M. haemolytica* vaccine significantly augmented immunity against the bacterium (Confer et al., 2003). In that study it was determined that vaccination with currently marketed commercial *M. haemolytica* vaccines or OMP-enriched preparations stimulated only low levels of antibody to PlpE.

Approximately 35% of *M. haemolytica* isolates from shipping fever pneumonia are S2 or S6, and cross-serotype protection against *M. haemolytica* serotypes has been difficult to achieve using conventional vaccine preparations (Purdy et al., 1993; Sabri et al., 2000). Since PlpE-like proteins are present in S2 and S6, we undertook studies to determine how similar PlpE is among strains of *M. haemolytica* S1, S2, and S6 at the molecular and antigenic levels. Therefore, our objective in this study was to clone and sequence the *plpE* gene from seven strains of S1, seventeen strains of S2 and eight strains of S6.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Thirty three *M. haemolytica* strains were used in this study (Table 1). Twenty-five strains were isolated from lungs and nasal swabs of cattle and identified at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL, OSU, Stillwater, OK). Eight strains were kindly provided by Dr. Robert Briggs (USDA-ARS, National Animal Disease Laboratory, Ames, IA). Isolates were identified according to standard protocols (Quinn et al., 1994). Serotypes of *M. haemolytica* were determined with chicken antisera using a rapid slide agglutination test as described (Frank and Wessman, 1978). Well-characterized isolates were grown in brain heart infusion (BHI) broth and preserved in 50% glycerol at −70 °C. Frozen stock cultures were plated onto BHI agar plates supplemented with 5% sheep blood and grown at 37 °C in a 5% CO_2 environment for 18 h. Isolated colonies from each were transferred into culture tubes each containing 5 ml BHI broth and incubated in a 37 °C shaker/incubator at 120 rpm to mid log phase.

2.1.1. Preparation of genomic DNA

Cells from 1.5 to 3.0 ml of mid log phase cultures were harvested by centrifugation in a bench top microfuge at 13,000 × *g*. Genomic DNA was extracted from each pellet as described (Ausubel et al., 1999) and stored at −20 °C.

2.1.2. PCR amplification and DNA sequencing

DNA fragments encoding the *plpE* gene were amplified with Taq DNA polymerase (Invitrogen, Carlsbad, CA) in polymerase chain reactions (PCR) using primer pairs shown in Table 2. Amplification of the expected size products was confirmed by agarose gel electrophoresis. Amplicons were purified with QIAquick PCR purification and Gel extraction kits (Qiagen, Chatsworth, CA) and submitted to the Oklahoma State University Recombinant DNA/Protein