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# Comparative functional genomic analysis of Pasteurellaceae adhesins using phage display

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**Abstract**

The Pasteurellaceae contain a number of important animal pathogens. Although related, the various members of this family cause a diversity of pathology in a wide variety of organ systems. Adhesion is an important virulence factor in bacterial infections. Surprisingly little is known about the adhesins of the Pasteurellaceae. To attempt to identify the genes coding for adhesins to some key components of the hosts extracellular matrix molecules, phage display libraries of fragmented genomic DNA from *Haemophilus influenzae*, *Actinobacillus pleuropneumoniae*, *Pasteurella multocida* and *Aggregatibacter actinomycetemcomitans*, were prepared in the phage display vector pG8SAET. The libraries were screened against human or porcine fibronectin, serum albumin or a commercial extracellular matrix containing type IV collagen, laminin and heparin sulphate. Four genes encoding putative adhesins were identified. These genes code for: (i) a 34 kDa human serum albumin binding protein from *Haemophilus influenzae*; (ii) a 12.8 kDa fibronectin-binding protein from *Pasteurella multocida*; (iii) a 13.7 kDa fibronectin-binding protein from *A. actinomycetemcomitans*; (iv) a 9.5 kDa serum albumin-binding protein from *A. pleuropneumoniae.* None of these genes have previously been proposed to code for adhesins. The applications of phage display with whole bacterial genomes to identify genes encoding novel adhesins in this family of bacteria are discussed.


*Keywords:* Phage display; Pasteurellaceae; Adhesins; Fibronectin

## 1. Introduction

The family Pasteurellaceae currently contains 61 taxa in 12 genera (www.the-icsp.org/taxa/Pasteurellaceaelist.htm). This family of bacteria contain a significant number of opportunistic pathogens and

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pathogens of humans and domesticated animals. Human pathogens include *Haemophilus influenzae* (St Geme, 2000, 2002; Rodriguez et al., 2003), *Haemophilus ducreyi* (Spinola et al., 2002), *Aggregatibacter actinomycetemcomitans* (Henderson et al., 2003) and *Pasteurella multocida* (Hunt et al., 2000). These organisms cause a range of diverse diseases such as meningitis, otitis media, chancroid, pneumonia, periodontitis and cat cuddlers cough, to name but a few. Animal pathogens include *P. multocida* (Hunt et al., 2000), *Actinobacillus pleuropneumoniae* (Bosse et al., 2002), *Actinobacillus equuli* (Rycroft and Garside, 2000) and *Mannheimia haemolytica* (Frank, 1989), which cause, among other diseases, atrophic rhinitis, fowl cholera, porcine pleuropneumonia, equine fatal septicaemia and bovine pneumonic pasteurellosis.

The ability of these related bacteria to cause such a diverse range of pathology is interesting and must reflect a diversity of virulence behaviors. One of the basic elements in bacterial pathogenesis is adhesion. There is now an enormous literature on the adhesins of a range of microorganisms (Ofek and Doyle, 1993) from Gram-negative enteric bacteria (Korhonen et al., 1982) to Gram-positive organisms such as the staphylococci (Peacock et al., 1999). However, relatively little is known about the adhesins used by the Pasteurellaceae to colonise their hosts and cause disease. In an attempt to identify, and to compare and contrast, the adhesins used by the Pasteurellaceae to colonise their hosts we have used phage display with the fragmented genomic DNA from four members of this family: *H. influenzae*, *P. multocida, A. pleuropneumoniae* and *A. actinomycetemcomitans*. The genomes of these organisms have been or are in the process of being sequenced so enabling the full-length open reading frames associated with the gene fragments identified by phage display to be determined.

## 2. Materials and methods

### 2.1. Bacterial strains and plasmids

*H. influenzae* NCTC 8470/ATCC 9332 Pittman type D and *P. multocida* NCTC 10322/ATCC 43137 (pig isolate) were purchased from the National Collection of Type Cultures (London, UK) and cultured on chocolate agar or grown in Brain Heart Infusion (BHI) broth (Oxoid Ltd., Basingstoke, United Kingdom) aerobically at 37 °C. BHI broth was supplemented with 10 μg/ml hemin and 2 μg/ml β-NAD (Sigma–Aldrich Co. Ltd., Poole, United Kingdom) in the case of *H. influenzae*. *A. pleuropneumoniae* serotype 1, strain 4074 was routinely cultured on chocolate agar, then grown in BHI broth supplemented with 2 μg/ml NAD, aerobically at 37 °C. *A. actinomycetemcomitans* strain HK1651 (JP2 clone) was maintained on blood agar or grown in BHI broth at 37 °C in a 5% $CO_2$ atmosphere. All of these strains are clinical isolates.

The phagemid pG8SAET (a kind gift from Lars Frykberg, Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden) and the *Escherichia coli* host TG1 [*supE hsd Δ*5 *thi* Δ (*lac-proAB*) F′(*traD36 proAB*$^+$ *lacI*$^q$ *lacZΔ*M15)] were used in the construction of the phage display library. *E. coli* was grown in nutrient broth no. 2 (NB-2, Oxoid Ltd.). The medium was supplemented when appropriate with 200 μg/ml of ampicillin to maintain the phagemid. All cultures of *E. coli* TG1 were grown at 37 °C under aerobic conditions.

### 2.2. Purification of porcine fibronectin from pig serum

Pig serum was purchased from Invitrogen–Gibco. Serum was diluted 1:5 in stabilization buffer (50 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA at pH 7.4) and applied to a 10 ml gelatin-Sepharose (Sigma–Aldrich) column equilibrated with stabilisation buffer. Bound proteins were eluted from the column with 20 ml of elution buffer (50 mM Tris–HCl, 250 mM NaCl, 1 mM EDTA, 3 M urea at pH 7.4). The eluant was then diluted 1:1 in stabilization buffer and applied to a 10 ml heparin-Sepharose (Sigma–Aldrich) column that had been extensively washed with stabilization buffer. After first washing the column with 40 ml of stabilisation buffer, bound proteins were eluted in 40 ml of elution buffer (50 mM Tris–HCl, 500 mM NaCl, 1 mM EDTA, at pH 7.4). The isolated fibronectin was analysed by SDS-PAGE to ensure it was at least 95% homogenous.