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Review

The pathogenesis of ovine footrot

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ABSTRACT

Ovine footrot is a contagious and debilitating disease that is of major economic significance to the sheep meat and wool industries. The causative bacterium is the gram negative anaerobe *Dichelobacter nodosus*. Research that has used a classical molecular genetics approach has led to major advances in our understanding of the role of the key virulence factors of *D. nodosus* in the disease process. *D. nodosus* strains produce polar type IV fimbriae and extracellular serine proteases. Mutagenesis of the fimbrial subunit gene *fimA* and the *pilT* gene, which is required for fimbrial retraction, and subsequent testing of these mutants in sheep virulence trials has shown that type IV fimbriae-mediated twitching motility is essential for virulence. The extracellular protease genes *aprV2, aprV5* and *bprV* have also been mutated. Analysis of these mutants has shown that ArpV5 is the major extracellular protease and that AprV2 is the thermostable protease that is responsible for the extracellular elastase activity. Structural analysis of AprV2 has revealed that it contains several novel loops, one of which appears to act as an exosite that may modulate substrate accessibility. Finally, virulence experiments in sheep have shown that the AprV2 protease is required for virulence.

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1. Introduction

Ovine footrot is a contagious and debilitating disease of the feet of sheep and is characterized by the separation of the keratinous hoof from the underlying tissue, which results in lameness and loss of body condition (Egerton et al., 1969; Stewart et al., 1984). Consequently, the disease has a significant financial impact on the world-wide sheep

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industry due to a reduction in meat and wool production and the expenditure associated with treatment and prevention programs (Green and George, 2008; Stewart, 1989; Wani and Samanta, 2006).

The severity of ovine footrot can vary from benign footrot, which presents as an interdigital dermatitis that does not progress, to virulent footrot, which results in severe underrunning of the horn of the hoof and the separation of the hoof from the underlying tissue (Stewart, 1989). The principle causative agent of ovine footrot is *Dichelobacter nodosus*, a slow-growing, anaerobic bacterium. The severity of disease is dependent on the nature of the particular isolate and climatic conditions, warm moist conditions and temperatures above 10 °C being essential for transmission (Whittington and Nicholls, 1995). The essential virulence factors of *D. nodosus* are its type IV fimbriae and the ability to produce extracellular serine proteases (Kennan et al., 2001, 2010). For many years, these extracellular proteases have been the basis of diagnostic tests that differentiate virulent and benign strains (Depiazzi and Richards, 1979; Palmer, 1993), with virulent strains generally having greater elastase activity and protease thermostability than benign strains. However, these tests are generally time consuming and often not definitive (Rood et al., 1996). More recent studies have observed that a genetic element that contains an *intA* gene was preferentially associated with virulent strains and it was suggested that an *intA*-specific PCR together with a gelatin-gel test for protease thermostability may provide a more definitive diagnosis of benign or virulent footrot (Cheetham et al., 2006).

Until about 10 years ago our knowledge of the role of putative virulence factors in the disease process was limited by an inability to genetically manipulate *D. nodosus*. We now know that many strains of *D. nodosus* are naturally transformable (Kennan et al., 1998, 2001, 2003) and we have developed genetic methods whereby allelic replacement can be used to generate isogenic chromosomal mutants of *D. nodosus* that can subsequently be tested in a sheep disease model. Therefore, it is now possible to test molecular Koch's postulates on any putative virulence gene. Although such experiments are very expensive to carry out, they have led to a very significant increase in our understanding of the role of type IV fimbriae and extracellular proteases in the disease process. In addition, the genome of the virulent, transformable isolate VCS1703A has been completely sequenced (Myers et al., 2007), confirming that *D. nodosus* has a small genome (1.4-Mb) and shows no evidence of ongoing genome reduction. While several other potential virulence factors were identified in this study (Calvo-Bado et al., 2011; Myers et al., 2007), their role in virulence is yet to be determined. In this mini-review we will summarize how reverse genetics of *D. nodosus* has been applied to the analysis of disease pathogenesis.

2. Type IV fimbriae

2.1. Fimbrial biogenesis

D. nodosus possesses long filamentous appendages on the cell surface, which are termed type IV fimbriae due to their polar location and the conserved structure of the major fimbrial subunit protein (Hobbs et al., 1991; Mattick et al., 1991; Ottow, 1975; Stewart, 1973; Walker et al., 1973). Type IV fimbriae are found in many different bacterial species and confer a flagella-independent motility termed twitching motility (Mattick, 2002). A great deal is known about the biogenesis, structure and functional role of type IV fimbriae, primarily from studies carried out in *Pseudomonas aeruginosa* and *Neisseria* spp. (Craig and Li, 2008; Craig et al., 2004).

D. nodosus fimbriae are highly immunogenic and are responsible for the K-agglutination reaction, which provides the basis for the classification of *D. nodosus* isolates into 10 major serogroups, A–I, and M (Claxton et al., 1983; Egerton, 1973; Ghimire et al., 1998). Based on structural variation within the major fimbrial subunit protein FimA and the genetic organization of the *fimA* gene region, *D. nodosus* isolates from these serogroups have been divided into two major classes (Billington and Rood, 1991; Elleman et al., 1984; Finney et al., 1988; Ghimire et al., 1998; Hobbs et al., 1991; Hoyne et al., 1989; Mattick et al., 1991). Class I isolates (serogroups A–C, E–G, I, and M) have the *fimB* gene, which is not required for fimbrial biogenesis (Kennan et al., 2001), downstream of *fimA* (Hobbs et al., 1991). Strains in class II (serogroups D and H) contain three genes, *fimC*, *fimD* and *fimZ*, all of which are of unknown function, adjacent to the *fimA* gene. In both classes, upstream of *fimA* there is an *aroA* gene, which encodes enolpyruvylshikimate-3-phosphate synthase. An ATP-dependent protease gene, *clpB*, is located downstream of *fimB* in class I isolates and of *fimZ* in class II isolates (Hobbs et al., 1991).

Major fimbrial subunits of both classes have highly conserved N-terminal regions, which contain an *N*-methylphenylalanine, a short positively charged leader sequence and a hydrophobic domain. Sequence conservation exists in the seven-amino acid leader sequence and up to residue 18 of the mature protein and high level similarity extends up to residue 34, with conservative or semi-conservative substitutions that are mostly, but not absolutely, class-specific (Mattick et al., 1991). Less similarity is observed from residues 35 to 60 and in the C-terminal region, while the remainder of the protein exhibits significant variation (Mattick et al., 1991). Although most *D. nodosus* isolates belong to class I (Hobbs et al., 1991), the fimbrial subunits of class II strains have more in common with those of *P. aeruginosa* and *Moraxella bovis*, especially the region between residues 35 and 60 and at the C-terminus, where there is a disulphide bond that has been found in all type IV fimbrial subunits analyzed, except for *D. nodosus* class I subunits (Dalrymple and Mattick, 1987; Mattick et al., 1991).

Bioinformatic analysis revealed that 20 potential fimbrial biogenesis genes and 10 putative fimbrial regulatory genes were located at several different loci on the *D. nodosus* genome (Fig. 1) (Myers et al., 2007). These gene regions include the *fimNOP* locus (Han et al., 2007; Johnston et al., 1995), the *pilMNOPQ* locus, the *pilT-pilU* locus (Han et al., 2008), the *pilC* (related to *pilY1*) and pilin-like gene locus, *pilCVWXfimUpilE* (Han et al., 2007), the *pilSR* locus (Parker et al., 2006) and the *chpApilGHIJ* locus, which is potentially involved in PilT/U regulation (Bertrand