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Structural characterisation of the virulence-associated protein VapG from the horse pathogen *Rhodococcus equi*

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ABSTRACT

Virulence and host range in *Rhodococcus equi* depends on the variable pathogenicity island of their virulence plasmids. Notable gene products are a family of small secreted virulence-associated proteins (Vaps) that are critical to intramacrophagic proliferation. Equine-adapted strains, which cause severe pyogranulomatous pneumonia in foals, produce a cell-associated VapA that is necessary for virulence, alongside five other secreted homologues. In the absence of biochemical insight, attention has turned to the structures of these proteins to develop a functional hypothesis. Recent studies have described crystal structures for VapD and a truncate of the VapA orthologue of porcine-adapted strains, VapB. Here, we crystallised the full-length VapG and determined its structure by molecular replacement. Electron density corresponding to the N-terminal domain was not visible suggesting that it is disordered. The protein core adopted a compact elliptical, anti-parallel β-barrel fold with β1–β2–β3–β8–β5–β6–β7–β4 topology decorated by a single peripheral α-helix unique to this family. The high glycine content of the protein allows close packing of secondary structural elements. Topologically, the surface has no indentations that indicate a nexus for molecular interactions. The distribution of polar and apolar groups on the surface of VapG is markedly uneven. One-third of the surface is dominated by exposed apolar side-chains, with no ionisable and only four polar side-chains exposed, giving rise to an expansive flat hydrophobic surface. Other surface regions are more polar, especially on or near the α-helix and a belt around the centre of the β-barrel. Possible functional significance of these recent structures is discussed.

1. Introduction

Rhodococcus equi is a ubiquitous, soil bacterium which has evolved into an opportunistic pathogen through the acquisition of an 80 kbp virulence plasmid. Pathogenic *R. equi* infect different domesticated animal hosts (pigs, sheep and cattle) but are most frequently associated with bronchopneumonial disease in very young foals. It is also an emerging human pathogen, affecting immuno-compromised individuals. In foals, disease is transmitted through inhalation of contaminated dust particles, leading to severe lung infection which eventually spreads to the gut and other areas (Meijer and Prescott, 2004; Vázquez-Boland et al., 2013). Excretion of contaminated faeces completes the infection cycle. Clinical manifestations of *R. equi* lung infection include bronchitis and pulmonary lesions (Prescott, 1991), and in the absence of treatment mortality rates are in the region of 80% (Muscatello et al., 2007). Thus, *R. equi* represents a major threat to foal health

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worldwide and has a significant economic impact on the horse breeding industry. Current treatments for *R. equi* infections involve combination drug therapies with rifampin and macrolides such as clarithromycin (Giguère et al., 2011). These treatments can be protracted and expensive, and are not always successful. Furthermore, antibiotic resistant strains are emerging (Andersen et al., 1997; Asoh et al., 2003). While vaccine trials are on-going and making headway (Hooper-McGrevy et al., 2005; Dawson et al., 2010; Whitehead et al., 2012), there are still no commercially available vaccines, and research investigations are now focussing on the mechanism of pathogenicity induced by *R. equi* in order to provide insights which may ultimately lead to better treatments for infection.

R. equi is a Gram-positive coccobacillus with a lipid-rich cell envelope characteristic of the mycolata, a group of acid-fast actinomycetes that includes *Mycobacterium, Corynebacterium* and *Nocardia* species. It metabolises a wide range of organic and inorganic compounds and thrives on the mixture of organic compounds found in animal manure. Virulent *R. equi* enter alveolar macrophages by receptor-mediated phagocytosis and survive and proliferate in phagosomes. The bacterium has the ability to halt phagosome maturation prior to the late endocytic stage, thereby protecting it from normal bacteriocidal factors and allowing it to proliferate (Fernandez-Mora et al., 2005). Virulence of *R. equi* strains is strictly associated with the possession of an ~80 kbp plasmid (Takai et al., 1991b; Tkachuk-Saad and Prescott,

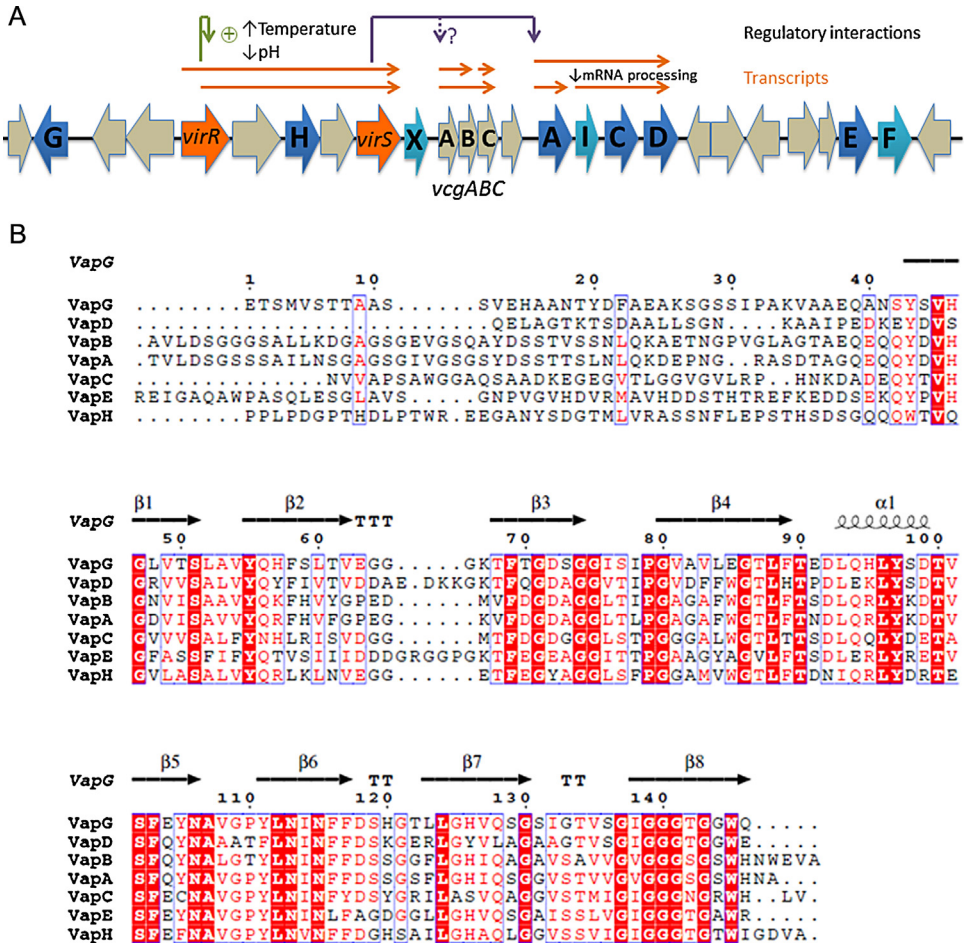

Fig. 1. The virulence genes/proteins of *R. equi*. (A) The 21.3 kb pathogenicity island region of the equine type *R. equi* plasmid pVAPA1037. The genes encoding the homologous virulence associated proteins Vaps A, C, D, E, G, H are shown in blue with a one letter label. The pseudogenes *vapF*, *vapI* and *vapX* are shown in turquoise. The genes encoding the transcriptional regulators *virR* (a LysR type transcriptional regulator) and *virS* (an orphan two-component system response regulator) are coloured in orange. Expression of *vapA* appears to be controlled indirectly by VirR in response to temperatures above 30 °C and acidic pH by production of VirS (green arrow) (Russell et al., 2004), which is required for transcription of the *vapAIC(orfAB)D* operon (purple arrow) (Kakuda et al., 2014); the small uncharacterised ORFs (*orfAB*) that lie between *vapC* and *vapD* are not shown for clarity. The promoter associated with the *vcgABC* operon (uncharacterised *vap* co-expressed genes) shares homology with the *vapA* promoter (Miranda-CasoLuengo et al., 2011) suggesting that a similar mode of regulation may operate. Observed transcripts are related as orange arrows (Byrne et al., 2007, 2008; Kakuda et al., 2014; Miranda-CasoLuengo et al., 2011). (B) Comparison of the amino acid sequences of the Vap proteins of the *R. equi* horse virulence plasmid together with VapB form the porcine virulence plasmid. The sequences were aligned in CLUSTALW (Thompson et al., 1994) and displayed together with the secondary structure elements of VapG using the programme ESPRIPT (Robert and Gouet, 2014). Invariant residues in the alignment are shown in white type on a red background; conserved residues are in blue boxes (For interpretation of the colour information in this figure legend, the reader is referred to the web version of the article.).