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Structure and genotypic plasticity of the *Campylobacter fetus sap* locus

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Summary

The *Campylobacter fetus* surface layer proteins (SLPs), encoded by five to nine *sapA* homologues, are major virulence factors. To characterize the *sapA* homologues further, a 65.9 kb *C. fetus* genomic region encompassing the *sap* locus from wild-type strain 23D was completely sequenced and analysed; 44 predicted open reading frames (ORFs) were recognized. The 53.8 kb *sap* locus contained eight complete and one partial *sapA* homologues, varying from 2769 to 3879 bp, sharing conserved 553–2622 bp 5′ regions, with partial sharing of 5′ and 3′ non-coding regions. All eight *sapA* homologues were expressed in *Escherichia coli* as antigenic proteins and reattached to the surface of SLP$^-$ strain 23B, indicating their conserved function. Analysis of the *sap* homologues indicated three phylogenetic groups. Promoter-specific polymerase chain reactions (PCRs) and *sapA* homologue-specific reverse transcription (RT)-PCRs showed that the unique *sapA* promoter can potentially express all eight *sapA* homologues. Reciprocal DNA recombination based on the 5′ conserved regions can involve each of the eight *sapA* homologues, with frequencies from 10^{-1} to 10^{-3}. Intragenic recombination between *sapA7* and *sapAp8*, mediated by their conserved regions with a 10^{-1}–10^{-2} frequency, allows the formation of new *sap* homologues. As divergent SLP C-termini possess multiple antigenic sites, their reciprocal recombination behind the unique *sap* promoter leads to continuing antigenic variation.

Introduction

Campylobacter fetus are spiral, microaerophilic, Gram-negative bacterial pathogens that cause infertility and infectious abortion in ungulates, and septicaemia, meningitis and other systemic infections in humans, especially in infants and HIV-infected persons (Guerrant *et al*., 1978; Garcia *et al*., 1983; Skirrow, 1990; Blaser, 1998; Thompson and Blaser, 2000). In common with many other bacteria (Walker *et al*., 1992; Sleytr *et al*., 1993), *C. fetus* expresses a paracrystalline surface layer (S-layer) on its outermost cell surface (Dubreuil *et al*., 1988; 1990; Fujimoto *et al*., 1991). The S-layer is the major *C. fetus* virulence factor, rendering cells resistant to serum killing by impairing C3b binding (Blaser *et al*., 1987; 1988; Blaser and Pei, 1993). Each S-layer is composed of high-molecular-weight S-layer proteins (SLPs), and *C. fetus* cells vary the SLPs expressed. The SLPs are essential for host colonization (Grogono-Thomas *et al*., 2000), and their antigenic variation helps to evade host immune responses (Wang *et al*., 1993; Garcia *et al*., 1995).

In culture, each *C. fetus* strain usually expresses one predominant SLP, although subpopulations of cells can express variant SLPs of apparent molecular weights ranging from 97 to 149 kDa (Blaser *et al*., 1994). The SLPs are encoded by five to nine *sapA* homologues tightly clustered on the chromosome (Dworkin *et al*., 1995a; Garcia *et al*., 1995; Tu *et al*., 2001a), and each *sapA* homologue is potentially expressed by the unique *sapA* promoter (Dworkin and Blaser, 1996). SLP phenotypic switching in *C. fetus* appears to involve high-frequency chromosomal DNA rearrangements that occur within the *sap* genomic locus, as shown in studies of three *sapA* homologues (Dworkin *et al*., 1997; Dworkin and Blaser, 1997a; Ray *et al*., 2000; Tu *et al*., 2001b).

As the genomic organization and the structural features of all the *sap* genes have not been described, we have now identified and characterized a 53.8 kb chromosomal region containing the entire *sap* locus in wild-type strain 23D. We show that all eight complete *sapA* homologues share conserved regions at their 5′ regions, encode SLPs from 96 kDa to 131 kDa that share similar characteristics and can be divided into three phylogenetic groups based

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