

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

Zheng-Chao Tu,¹ Trudy M. Wassenaar,²
Stuart A. Thompson³ and Martin J. Blaser^{1,4*}

¹Division of Infectious Diseases, Department of Medicine,
New York University School of Medicine, 550 First Avenue,
New York, NY 10016, USA.

²Molecular Microbiology and Genomics Consultants,
Zotzenheim, Germany.

³Department of Biochemistry and Molecular Biology,
Medical College of Georgia, Augusta, GA, USA.

⁴Department of Veterans Affairs Medical Center, New
York, NY, USA.

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 *sapA8*, 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 recombina-

tion 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

Accepted 13 January, 2003. *For correspondence. E-mail martin.blaser@med.nyu.edu; Tel. (+1) 212 263 6394; Fax (+1) 212 263 7700.