Physical and genetic map of the genome of *Campylobacter upsaliensis*

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A physical map of *Campylobacter upsaliensis* ATCC 43954 was constructed from DNA fragments generated by *SalI* (5' G/TCGAC), *NarI* (5' GG/CGCC) and *BssHII* (5' G/CGCGC) restriction digests separated using pulsed-field gel electrophoresis. The size of the *C. upsaliensis* genome was approximately 2000 kb, providing evidence of the largest *Campylobacter* genome sized to date. Twenty-one fragments created from these restriction digests were assembled into a physical map using a combination of complementary methods including cross-Southern hybridization, hybridization fingerprint analysis and hybridization with homologous and heterologous (from *Campylobacter jejuni*) gene probes. The position of ten genetic loci, including that of the iron-uptake regulatory (*fur*) gene, were localized to the physical map. A genomic library of *C. upsaliensis* ATCC 43954 was constructed in lambda Gem-11 vector. Fifty thousand recombinants with an average size of 16 kb represent a library about 200 times the size of the genome. Using *C. jejuni* DNA probes, clones representing *C. upsaliensis* *flaA*, *fur* and *ftsZ* genes were isolated and localized to the physical map.

Keywords: *Campylobacter*, genomic map

INTRODUCTION

Campylobacter upsaliensis is increasingly recognized as an important human enteropathogen (Sandstedt *et al.*, 1983; Lastovica *et al.*, 1989; Taylor *et al.*, 1989; Walmsley & Karmali, 1989). *C. upsaliensis* is associated with acute self-limiting diarrhoea but it has also been isolated in the setting of chronic diarrhoea and failure to thrive in infants (Megraud & Bonnet, 1986). Moreover, *C. upsaliensis* can cause bacteraemia in debilitated and immuno-compromised patients (Patton *et al.*, 1989; Chusid *et al.*, 1990). In a recent report (Steele *et al.*, 1985), this thermophilic, catalase-negative *Campylobacter* species comprised more than one-quarter of all faecal *Campylobacter* isolates in children under 3 years old. Some reports indicate that *C. upsaliensis* is a more frequent human faecal isolate than *Campylobacter coli* (Steele *et al.*, 1985; Goossens *et al.*, 1990). Thus, *C. upsaliensis* may be the second most commonly identified human intestinal campylobacter after *Campylobacter jejuni*.

Despite its importance in human disease, little is known about the virulence properties of *C. upsaliensis* (Sandstedt

& Ursing, 1991). Furthermore, the organism remains to be characterized using a molecular biological approach. Genetic characterization of *C. upsaliensis* is an essential step towards understanding at a molecular level the role of this bacterium in human disease.

Pulsed-field gel electrophoresis (PFGE) technology has proven invaluable for the accurate estimation of genome size (Lee & Smith, 1988; Pyle *et al.*, 1988) and in the construction of physical maps for a diverse range of prokaryotic organisms (Smith *et al.*, 1987; Romling *et al.*, 1989; Allerdet-Servent *et al.*, 1990; Ladefoged & Christiansen, 1992; Alleman *et al.*, 1993; Murray *et al.*, 1993; Neumann *et al.*, 1993). Physical maps exist for several *Campylobacter* species including *C. jejuni* (Chang & Taylor, 1990; Nuijten *et al.*, 1990; Kim *et al.*, 1992), *C. coli* (Yan & Taylor, 1991) and *C. fetus* (Salama & Taylor, 1993). Despite its importance as a human enteric pathogen, a genomic map has yet to be developed for *C. upsaliensis*. The generation of a physical and genetic map of *C. upsaliensis* reported in this manuscript will help direct future gene-cloning and fine-mapping strategies for this organism.

METHODS

DNA preparation. The type strain of *C. upsaliensis* (ATCC 43954/NCTC 11541; American Type Culture Collection, Rockville, MD, USA) was grown at 37 °C in 7% (v/v) CO₂ in Brucella broth supplemented with 10% (v/v) foetal calf serum. To obtain adequate overnight growth, small broth culture volumes were necessary; typically 20–30 ml cultures per 500 ml Erlenmeyer flask. Genomic DNA was prepared in agarose plugs by previously described methods (Smith *et al.*, 1988; Maslow *et al.*, 1993). Briefly, bacterial cultures were centrifuged, washed and resuspended in 10 mM Tris/HCl (pH 7.6)/1 M NaCl to which was added an equal volume of 1.3% (w/v) low-melting-point agarose (BRL). The resuspended bacteria were then aliquoted to precooled 0.1 ml plug moulds (Pharmacia) and allowed to solidify at 4 °C for 30 min. Spheroblasting and deproteinization were undertaken as described by Smith *et al.* (1988). Prior to restriction enzyme digestion, inserts were washed in 3 × 10 ml aliquots of 10 mM Tris/HCl (pH 7.5)/1 mM EDTA (TE) at 37 °C for a minimum of 2 h per wash.

Digestion with restriction enzymes was performed in a total volume of 0.3 ml, with 20–30 U of enzyme per insert. *Sma*I, *Sal*I and *Bss*HII (Boehringer Mannheim) digests were incubated overnight at 25 °C, 37 °C and 50 °C, respectively. *Nar*I (BRL) restriction enzyme digests were incubated for 7 h at 37 °C to avoid DNA degradation observed under more prolonged incubation conditions.

Electrophoresis. PFGE was undertaken using a contour-clamped homogeneous-electric field (CHEF) apparatus with programmable, autonomously controlled digital/analogue converters at each electrode (PACE system) (LKB 2015 Pulsaphor gel electrophoresis unit and pulse time controller; Pharmacia LKB Biotechnology). DNA samples were loaded onto 1% agarose (ICN Biomedicals) and run in 0.5 × TBE buffer (Sambrook *et al.*, 1989) at 14 °C. Optimal resolution of the *Sal*I, *Nar*I and *Bss*HII restriction fragments used to construct the physical map of *C. upsaliensis* was achieved using pulse times linearly ramped from 5 to 45 s over the course of 21 h with a field strength of 10 V cm⁻¹. Lambda concatemers (Promega) and the chromosomes of *Saccharomyces cerevisiae*, strain YPH149 (Kim *et al.*, 1992), were used as size markers. For the purposes of hybridization fingerprint analysis, *C. upsaliensis* *Sma*I restriction digests were resolved in 1.2% agarose using pulse times linearly ramped from 2 to 5 s for 24 h. Gels were stained for 30 min in ethidium bromide (0.5 mg ml⁻¹), destained overnight and photographed under ultraviolet light.

Southern hybridization. Chromosomal DNA fragments from pulsed-field gels were transferred onto GeneScreen Plus nylon membrane (Dupont-NEN) using a Vacugene apparatus (Pharmacia). The gel was depurinated in 0.25 M HCl, denatured in 0.5 M NaOH/0.5 M NaCl, neutralized in 1 M Tris (pH 7.5)/1.5 M NaCl and transferred in 20 × SSC [3 M NaCl/0.3 M sodium citrate (pH 7)].

Library preparation. High molecular mass genomic DNA was isolated from *C. upsaliensis* ATCC 43954 according to the procedure of Thomas *et al.* (1966). Following extraction, purification and partial digestion with *Sau*3A1, the DNA was fractionated through a sucrose gradient and fragments in the size range 9–23 kb were isolated (Sambrook *et al.*, 1989). This DNA was then ligated into phage lambda GEM-11 *Bam*HI arms (Promega) and recombinants were packaged with Packagene Extracts (Promega). After titration, the library was estimated to contain 2.5 × 10⁸ recombinant clones. With an average insert size of 16 kb, the library is, thus, approximately 200 times the genome size. Nine hundred and sixty individual phage plaques

were harvested and stored in 96-well titre plates. Phage DNA was harvested from individual clones as described by Grossberger (1987). Aliquots of each recombinant phage clone from the lambda library were blotted (Hybridot Manifold, BRL) to nylon membrane (GeneScreen) for subsequent dot-blot hybridization. DNA fragments used to probe blots of the genomic digests and to screen the lambda library were labelled with [α -³²P]dATP (ICN Biomedicals) by nick translation (Sambrook *et al.*, 1989) and hybridized to the GeneScreen Plus membrane, using conditions recommended by the manufacturer.

RESULTS

Electrophoretic analysis and genome size

The restriction enzymes *Sal*I (5' G/TCGAC), *Nar*I (5' GG/CGCC) and *Bss*HII (5' G/CGCGC) each cleaved the *C. upsaliensis* genome into an appropriate number of fragments for use in generating a physical map of the organism. *Sal*I, *Nar*I and *Bss*HII produced 5, 8 and 10 restriction fragments, respectively (Table 1, Fig. 1). *Nru*I (5' TCG/GCA), *Xho*I (5' C/TCGAG), *Bgl*I (5' GCCN₄/NGGC), and two restriction enzymes found to be useful for mapping other *Campylobacter* species (Kim *et al.*, 1992), *Sma*I (5' CCC/GGG), and *Sac*II (5' CCGC/GG), cleaved the *C. upsaliensis* genome too frequently to be of use for macrophysical mapping. *Apa*I (5' GGG/CCC) and *Sfi*I (5' GGCCN₄/NGGCC) did not cleave the *C. upsaliensis* genome (data not shown).

The size of the *C. upsaliensis* ATCC 43954 genome was estimated at 2007 kb by adding together the sizes of the fragments from the *Sal*I, *Nar*I and *Bss*HII digests and

Table 1. PFGE fragment sizes for *C. upsaliensis* ATCC 43954

Fragment sizes represent the mean of four independent PFGE runs. Electrophoresis conditions were altered during these runs. All 16 yeast chromosomes were fully resolved during one run to ensure accurate size estimation for higher molecular mass restriction fragments. The mean genome size for *C. upsaliensis* is 2007 ± 67.5 kb (± standard deviation).

Fragment	Fragment size (kb)		
	<i>Sal</i> I	<i>Nar</i> I	<i>Bss</i> HII
A	665	560	490
B	615	410	420
C	385	365	320
D	190	230	285
E	85	165	165
F		125	130
G		88	85
H		63	65
I			60
J			55
Total	1940	2006	2075

Fig. 1. PFGE gel of *C. upsaliensis* DNA. PFGE fragments are indicated alphabetically on the right and molecular sizes on the left side of each gel. Gels were run at 12–14 °C in a field strength of 10 V cm⁻¹. (a) *SalI* digest of *C. upsaliensis* DNA (lane 3). A 1% agarose gel was run for 21 h using a pulse time linearly ramped from 5 to 45 s. Lambda concatemers (lane 1) and yeast chromosomes (lane 2) were used as size markers. (b) *NarI* digest of *C. upsaliensis* DNA. Electrophoresis conditions were as for (a). The unresolved, high molecular mass, strongly staining band in the *NarI* digest (○) represents uncut genomic DNA. Some variation in PFGE band intensity is seen. This probably represents differences in the efficiency of cleavage of *NarI* recognition sites in the genomic DNA. *NarI* H is not visible in this digest. (c) *BssHII* digest of *C. upsaliensis* DNA. A 1.1% gel was run for 30 h using a pulse time linearly ramped from 5 to 50 s. Three fainter bands representing partially digested DNA are indicated by asterisks. Some of the smaller *BssHII* fragments are not well visualized on this gel.

Fig. 2. Southern hybridization of a PFGE gel of *C. upsaliensis* ATCC 43954 probed with *BssHII* G fragment. The gel was run for 21 h using a pulse time linearly ramped from 5 to 45 s and a field strength of 10 V cm⁻¹. Molecular sizes are indicated to the left of the blot. Lanes 1–3 indicate *BssHII*, *NarI* and *SalI* digests, respectively. The black arrowhead indicates *BssHII* G, *NarI* G and *SalI* E bands identified by the radiolabelled probe. The open arrowhead indicates anomalous hybridization to the *BssHII* H fragment.

taking the mean value from four independent PFGE runs utilizing different electrophoresis conditions (Table 1).

Physical and genetic mapping of the genome

Three complementary methods were employed to align restriction fragments from *SalI*, *NarI* and *BssHII* restriction digests into a physical map of the *C. upsaliensis* genome.

(i) Cross-Southern hybridization. Individual bands from each restriction digest were isolated and purified from agarose gels (GeneClean; Bio 101) and labelled with [α -³²P]dATP. These ³²P-labelled pulsed-field fragments were then used to probe blots of genomic digests created by the other restriction enzymes (Fig. 2) (Kim *et al.*, 1992; Ladefoged & Christiansen, 1992; Neumann *et al.*, 1993).

Cross-Southern hybridization using *SalI* fragments as probes allowed unambiguous localization of the majority of *BssHII* and *NarI* restriction fragments and suggested a preliminary construct of the physical map. With *SalI* restriction fragments arranged as the outermost ring, regional arrangements of fragments from the three restriction digests were, for the most part, not difficult to infer. However, some ambiguous data accrued from the cross-hybridization experiments. The majority of anomalous cross-hybridizations were probably the consequence of random DNA degradation (Lee *et al.*, 1989) or non-specific endonucleases contaminating commercial restriction enzyme preparations (Allerdet-Servent *et al.*, 1991). For instance, *SalI* C and *NarI* G hybridized to each other. However, other restriction fragments that aligned with

Table 2. PFGE fragment cross-Southern hybridization patterns for *C. upsaliensis* ATCC 43954

For clarity, inconsistent cross-hybridization data have been omitted (discussed in the text).

Probe	Fragment identified		
	<i>SalI</i>	<i>Bss</i> HII	<i>NarI</i>
<i>SalI</i>			
A		C, D, E, G	A, G
B		A, B	B, C
C		B, C	C, D, E, F
D		B	C
E		G, H	G, H
<i>Bss</i>HII:			
A	B		B*
B	B, C, D		C, D
C	A, C	D†	A, E, F
D	A	C†	A
E	A, C		A
G	E	H	G
<i>NarI</i>:			
A	A	C, D, E	
B	B	A	
D	C, E	B, C	
G	A, E	G, C	

* Unexpectedly, *Bss*HII A did not hybridize to the *NarI* C fragment (see text).

† *Bss*HII C and *Bss*HII D mapped strongly to each other, suggesting the presence of a large repetitive sequence in this region of the chromosome (see text).

NarI G (i.e. *SalI* E and *Bss*HII G) did not cross-hybridize with *SalI* C. A number of other inconsistent hybridizations of this nature, mainly involving other *Bss*HII and *NarI* fragments, were also noted. DNA degradation was a feature of restriction enzyme digestion with both *NarI* and *Bss*HII and probably accounts for the frequency of these inconsistent hybridizations in this study. In fact, as a consequence of DNA degradation among the smaller *NarI* and *Bss*HII fragments, only *NarI* G and *Bss*HII G were useful as probes during cross-Southern experiments. The *NarI* G and *Bss*HII G fragments were preferentially cleaved during digestion of some DNA preparations and could be isolated from agarose in quantities large enough to ensure a favourable signal-to-noise ratio during probing.

Because the unexpected hybridizations outlined above were only observed between selected fragments in each chromosomal location, they cannot be explained on the basis of hybridization to homologous multicopy genes occurring in different chromosomal locations (Neumann *et al.*, 1993). However, the presence of homologous multicopy genes probably provides the explanation for some of the other ambiguous hybridization patterns. For instance, a large area of DNA homology provides a likely

Fig. 3. (a) *Smal* hybridization fingerprint for *SalI* C and *SalI* D fragments. *Smal*-digested *C. upsaliensis* DNA was resolved in 1.2% agarose using pulse times linearly ramped from 2 to 5 s for 24 h. Southern blots 1 and 2 indicate *Smal* digests probed with *SalI* C and *SalI* D fragments, respectively. Molecular sizes are indicated between the blots. (b) Southern blot of *SalI* digest probed with *Smal* 'a-e' band, a putative linking fragment for *SalI* A and E previously identified by hybridization fingerprinting. The *SalI* digest was resolved in 1% agarose run for 21 h using a pulse time linearly ramped from 5 to 45 s and a field strength of 10 V cm⁻¹. *SalI* fragments that hybridized with *Smal* 'a-e' are indicated by arrows. Molecular sizes are indicated to the left of the blot.

explanation for the strong cross-hybridization between *Bss*HII C and *Bss*HII D. Although neither the 16S nor 23S rRNA genes hybridized to *Bss*HII D, a number of other conserved gene markers hybridized with equal intensity to both of these loci (Table 3). This indicates the likelihood that this area of the genome contains an extensive duplication.

On the basis of fragment sizes, some overlap would be expected between *Bss*HII C and *NarI* D and also between *Bss*HII A and *NarI* C. Unexpectedly, cross-hybridizations did not confirm these overlaps. There are a number of possible explanations for this finding. Unfavourable signal-to-noise ratios may have obscured the result due either to small regions of overlap between two restriction fragments relative to their total size (Neumann *et al.*, 1993) or to relatively inefficient DNA labelling in the regions of overlap because of local DNA configurations. Alternatively, some smaller, as yet unmapped, DNA fragments may ultimately 'fill in' these apparent gaps. In this respect it should be noted that *Bss*HII F, *Bss*HII I and *Bss*HII J have not yet been placed onto this map.

Table 3. Assignment of genetic markers to the physical map of *C. upsaliensis* ATCC 43954

Probe	Gene function	Fragment identified			Source
		<i>SalI</i>	<i>NarI</i>	<i>BssHII</i>	
Homologous probes					
<i>glyA</i>	Serine hydroxymethyltransferase	D	C	B	S. T. Al Rashid & V. L. Chan (unpublished)
<i>λubfr3-1*</i>	Lambda library phage clone	D	C	B	This work
<i>λubf1-1*</i>	Lambda library phage clone	B	B	A	
<i>λubfz1-1*</i>	Lambda library phage clone	A	A	C, D†	
Heterologous probes					
(<i>C. jejuni</i>)					
<i>flaA</i>	Flagellin A subunit	A, B‡	A, B‡	E, A‡	Khawaja <i>et al.</i> (1992)
<i>figG</i>	Flagellar basal body rod protein	A	A	C, D†	Kim <i>et al.</i> (1993)
<i>figF</i>	Flagellar basal body rod protein	A	A	C, D†	H. Louie & V. L. Chan (unpublished)
<i>ftsZ</i>	Cell division protein	A	A	C, D†	
<i>fur</i>	Iron-uptake regulatory protein	D	C	B	V. L. Chan and others (unpublished)
<i>ileS</i>	Isoleucine-tRNA synthetase	A	A	C	Hong <i>et al.</i> (1995)
<i>lysS</i>	Lysyl-tRNA synthetase	D	C	B	Chan & Bingham (1992)
<i>trxB</i>	Thioredoxin reductase	C	F	C	H. Louie & V. L. Chan (unpublished)
60 kDa	Unknown	C	D	B	Y. Hong and others (unpublished)
16S rRNA	Ribosomal RNA	A, B, C	A, B, C, D, G	A, C, E	Kim <i>et al.</i> (1993)
23S rRNA	Ribosomal RNA	A, B, D	A, B, C	A, B, C	

* *λubfr3-1*, *λubf1-1* and *λubfz1-1* were identified by *C. jejuni fur*, *flaA* and *ftsZ* probes, respectively.

† Probes which hybridized to two separate *BssHII* fragments within the *SalI* A/*NarI* A fragments, suggesting the presence of a chromosomal duplication in this region.

‡ In addition to the *BssHII* E/*SalI* A/*NarI* A locus, *flaA* probed less strongly to *BssHII* A/*SalI* B/*NarI* B, C fragments (see text for discussion).

Despite apparent anomalies during cross-hybridization, this mapping technique functioned well as the primary tool in generating the physical map. The positions of the majority of restriction fragments could be correctly inferred using this mapping method alone. These alignments were then confirmed by using the complementary strategies detailed below.

(ii) Hybridization fingerprint analysis. *SmaI* cleaved the *C. upsaliensis* genome into more than 25 fragments, a number too great to be of use for physical mapping. However, this restriction enzyme was useful for hybridization fingerprinting (Neumann *et al.*, 1993). Radiolabelled *SalI* restriction fragments were used to probe *SmaI* digests of *C. upsaliensis* DNA. By comparing hybridization signal patterns generated by the individual *SalI* restriction fragment probes it was possible to identify *SmaI* fragments which linked the *SalI* fragments (Fig. 3a). This approach helped confirm the alignment of the *SalI* fragments in relation to each other.

(iii) Southern hybridization of genetic markers. Nine genetic markers previously cloned and sequenced in *C. jejuni*

strain TGH9011 (ATCC 43431) (Chan & Bingham, 1992; Khawaja *et al.*, 1992; Kim *et al.*, 1993; H. Louie & V. L. Chan, unpublished data; Y. Hong and others, unpublished data) were used as probes to create a genetic map of the *C. upsaliensis* genome (Table 3). Fragments of the encoding regions of each of these genes were hybridized to digests of *C. upsaliensis* DNA, thereby defining these genetic loci on the *C. upsaliensis* chromosome. In addition, the *flaA* (Khawaja *et al.*, 1992), *ftsZ* (H. Louie & V. L. Chan, unpublished data) iron-uptake regulatory (*fur*) (V. L. Chan and others, unpublished data) genes of *C. jejuni* were used to screen the lambda library for recombinant clones containing the homologous genes in *C. upsaliensis*. Single clones representing each of these probes, and the *C. upsaliensis* serine hydroxymethyltransferase (*glyA*) gene (S. T. Al Rashid & V. L. Chan, unpublished data), were used as homologous probes and localized to the map (Fig. 4). Although *C. upsaliensis* library clone *λubf1-1* was identified by a *C. jejuni flaA* probe, *λubf1-1* hybridized to a chromosomal locus identified only weakly by *flaA* (i.e. *SalI* B/*BssHII*/*NarI* B, C). This probably indicates the presence of a gene

Fig. 4. The physical map of *C. upsaliensis* ATCC 43954 generated with *SalI*, *BssHII* and *NarI*. Restriction fragments are designated alphabetically as indicated in the text. The location of genetic probes from *C. jejuni*, *C. upsaliensis* lambda library clones and the *SmaI* 'a-e' linking fragment are indicated.

homologous to *flaA* in this region. As expected, 16S and 23S ribosomal RNA genes of *C. jejuni* (Kim *et al.*, 1993) localized to multiple fragments on the physical map (Table 3). Therefore, the individual rRNA genes could not be unequivocally localized on the physical map of *C. upsaliensis*.

Construction of the map

Cross-Southern hybridization allowed confident alignment of the majority of restriction fragments. The left end of *SalI* D was aligned at 0° on the physical map. With *BssHII* B acting as linking fragment, *SalI* B was positioned adjacent to *SalI* D in a clockwise direction. *BssHII* A, *NarI* B and *NarI* C were aligned with these *SalI* fragments on the basis of cross-hybridization pattern and fragment size.

Working clockwise, *SalI* E defined a locus containing the G and H fragments of *BssHII* and *NarI* digests. This alignment was based on strong reciprocal hybridizations between these fragments. The possible alternative of placement of the *SalI* E locus between *SalI* A and *SalI* C was resolved by assuming that the unambiguous cross-hybridization pattern generated by *BssHII* G (Fig. 2) was correct. In this case, the alternative arrangement of this locus between *SalI* A and *SalI* C was untenable on the basis that *BssHII* G could not replace *BssHII* C as a linking fragment for *SalI* A and *SalI* C. This assumption was confirmed by hybridization with the *SmaI* 'a-e' fragment. *SmaI* 'a-e' hybridized to *SalI* E and *SalI* A (Fig. 3b),

indicating the contiguity of these two fragments. Furthermore, *SmaI* 'a-e' hybridized to *BssHII* G, *BssHII* H and *NarI* G but not *NarI* H (data not shown). This confirmed the alignment of *BssHII* G and *NarI* G fragments in relation to *SalI* E and documented the orientation of the G and H fragments of these digests in relation to adjacent restriction fragments (i.e. the H fragments both lie anticlockwise of the G fragments).

SalI A overlapped the G fragments of both *BssHII* and *NarI* digests and also hybridized to *BssHII* C, *BssHII* D, *BssHII* E and *NarI* A. The position of *NarI* A was apparent on the basis of its size and its failure to hybridize with other *SalI* fragments. *BssHII* C was known to be located at the clockwise extreme of *SalI* A because it served as a linking fragment with the adjacent *SalI* C fragment. *BssHII* D is located at the other extreme of *SalI* A as it was identified by the *SmaI* 'a-e' probe (data not shown). By inference, *BssHII* E must align between *BssHII* C and *BssHII* D in the centre of the *SalI* A and *NarI* A fragments. The position of the *BssHII* E fragment was then confirmed in this location by its hybridization with *flaA* from *C. jejuni*.

Completing the circle of the physical map, *SalI* C hybridized to *BssHII* C and *BssHII* B. The arrangement of *NarI* D clockwise to *NarI* E and *NarI* F was then inferred on the basis of hybridization patterns with *BssHII* B and *BssHII* C fragments.

Hybridization fingerprinting confirmed the contiguity of the *SalI* fragments inferred from cross-hybridization experiments (Fig. 3a). Localization of conserved genetic markers was then employed to support the alignment of restriction fragments on the physical map. Hybridization fingerprinting and hybridization with conserved genetic markers effectively eliminated those ambiguities encountered during cross-hybridization experiments.

DISCUSSION

We have constructed the first physical and genetic map of the genome of *C. upsaliensis*. This study also provides the first estimate of the genome size of this organism. To date, PFGE has been used to determine the genome sizes of three other *Campylobacter* species (Chang & Taylor, 1990; Nuijten *et al.*, 1990; Yan & Taylor, 1991; Kim *et al.*, 1992; Salama & Taylor, 1993), which vary from 1125 kb for *C. fetus* subsp. *fetus* (Salama & Taylor, 1993) to 1812 kb for *C. jejuni* (Kim *et al.*, 1992). At 2007 kb, *C. upsaliensis* is the largest campylobacter genome sized to date.

During PFGE of *C. upsaliensis* DNA, the presence of a number of partially digested *BssHII* and *NarI* fragments was a consistent feature of restriction digestion with these enzymes (Fig. 1b, c). More complete digestion of *C. upsaliensis* DNA with *BssHII* resulted in partial cleavage of the *BssHII* A fragment into 390 and 100 kb fragments. The presence of the 100 kb fragment was also sometimes noted on gels following standard *BssHII* restriction digestion. In addition, more complete digestion of *C. upsaliensis* DNA with *NarI* utilizing longer incubation times resulted in significant amounts of DNA degra-

Fig. 5. Comparison between the genomic maps of *C. upsaliensis* and *C. jejuni*. The outer circle represents the *Sall* restriction map of *C. upsaliensis* ATCC 43954. The inner circle represents the *Sall* restriction map of *C. jejuni* TGH9011 (Kim *et al.*, 1992). The two maps were aligned on the basis of *fla*, *glyA*, *lysS*, *fur* and 60 kDa gene loci, indicated in bold type. Genetic loci not sharing a common alignment on the two maps are indicated in small print.

dation. *NarI*-undigested DNA is seen on the gel as an unresolved, strongly staining, high molecular mass band (Fig. 1b). The *C. upsaliensis* type strain used in this study (ATCC 43954/NCTC 11541) was also resistant to digestion using a restriction enzyme (*HaeIII*) that was employed in a previous study (Owen & Hernandez, 1990).

The use of a number of complementary techniques during physical mapping of the *C. upsaliensis* genome facilitated the construction of a more detailed and accurate map. Cross-Southern hybridization patterns allowed preliminary alignment of the majority of restriction fragments (Table 2). Hybridization fingerprinting (Fig. 3a) and hybridization with genetic markers clarified ambiguities arising from unexpected cross-hybridizations between fragments positioned at different chromosomal locations.

Ambiguous hybridization patterns during cross-hybridization may be explained either by the presence of repetitive genes or operons in the *C. upsaliensis* genome (Neumann *et al.*, 1993) or by non-specific hybridization due to DNA degradation (Allardet-Servent *et al.*, 1991). DNA degradation presented a confounding variable during digestion using *NarI* and *BssHIII* and is, therefore, the likely cause of the majority of unexpected and inconsistent hybridization signals in this study. However, the presence of repetitive sequences in the genome of *C. upsaliensis* provides the likely explanation for some ambiguities encountered during the construction of the

physical map. In particular, cross-hybridization and localization of gene markers strongly suggests that the *C. upsaliensis* genome contains an extensive duplication generated through unequal cross-over between multicopy genes. This extensive duplication in the area of *BssHIII* C and *BssHIII* D could account for the observed large genome of *C. upsaliensis* relative to other campylobacters. This region of cross-homology does not appear to be due to the presence of a conserved rRNA operon as neither 16S rRNA nor 23S rRNA genes localize to the *BssHIII* D fragment (data not shown). The presence of extensive areas of cross-homology has not been noted during mapping studies of other *Campylobacter* species (Chang & Taylor, 1990; Nuijten *et al.*, 1990; Kim *et al.*, 1992).

A comparison of the genomic map of *C. upsaliensis* with that of *C. jejuni* TGH9011 is shown in Fig. 5. This comparison was based on five genetic loci (*fla*, *fur*, *lysS*, *glyA* and the 60 kDa gene), the relative positions of which were conserved on both maps. Future studies aimed at localization to the *C. upsaliensis* genomic map of other conserved genetic loci, including the origin of replication and rRNA genes, will be used to verify this alignment and to allow comparison with the genomic maps of other *Campylobacter* species (Nuijten *et al.*, 1990; Yan & Taylor, 1991; Salama & Taylor, 1993).

A primary objective of this mapping project was to localize potential virulence genes for *C. upsaliensis* to the genome. To this end, the genetic loci for the flagellin A subunit (*flaA*) and iron-uptake regulatory (*fur*), genes have been established on the *C. upsaliensis* physical map. A number of genetic loci representing genes serving other important physiological functions for this organism have also been identified. It is expected that with the identification and localization of other genes for *C. upsaliensis*, this genetic map will grow in complexity and function as a benchmark for further molecular biological studies of this emerging human enteropathogen.

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