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Size and physical map of the *Campylobacter jejuni* chromosome

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ABSTRACT

The chromosome of *Campylobacter jejuni* is circular and approximately 1700 kb in circumference. The size of the genome was determined by field inversion gel electrophoresis of restriction endonuclease fragments using lambda DNA concatamers and yeast chromosomes to calibrate the size of the fragments. In view of the low (32 – 35%) G+C content of the campylobacter genome, enzymes that recognizes GC-rich sequences were used. Of the enzymes tested *Bss*HII (G/CGCGC), *Nci*I (CC/SCG) and *Sal*I (G/TCGAC) appeared to be usable. Hybridization of labeled fragments with two or more fragments from digests with a different restriction enzyme gave the information to order the fragments on the *C.jejuni* chromosome. The localization on the genome of the flagellin and ribosomal gene clusters was determined.

INTRODUCTION

Campylobacter is a frequently occurring pathogenic bacterium causing severe diarrhea in humans (1). Yet, the unravelling of its pathogenicity on the molecular level has only recently begun. A few campylobacter genes have been cloned (1,2,3,4,5,6,7,8,9,10) and a method has been described to inactivate campylobacter genes by homologous recombination (11). Little is known about the campylobacter genome. Based on renaturation rate measurements a genomic size between 2.4 and 3.6×10^6 bp was determined (12). We have used field inversion gel electrophoresis (FIGE; 13) of restriction endonuclease fragments to determine the size of the genome of *C. jejuni* strain 81116 and Southern blot analysis to construct a physical map. Finally, we determined the location of the ribosomal and flagellin genes on the genomic map.

MATERIALS AND METHODS

Bacteria and plasmids

C. jejuni 81116 (obtained from D.G. Newell, PHLS, Salisbury, U.K.) was grown under microaerophilic conditions on saponine agar medium (5). As probes for the flagellin genes the 1.2 kb *Pst*I- *Bgl*II fragments of clone pIVB3-300 (6) were used. As a ribosomal probe we used a 1.5 kb *Bgl*II-*Hind*III fragment from clone pMYC202 of *Mycoplasma* P50, kindly provided by C. Christiansen (14).

Preparation of intact chromosomal DNA

Basically, the method described by McClelland et al. (15) was followed; bacteria from a 10 cm plate were harvested, washed with EET-solution (100 mM EDTA, 10 mM EGTA, and 10 mM Tris-HCl pH 8.0) and suspended in 2 ml 1% LMP agarose (Gibco/BRL) at 40°C. Agarose blocks (7×5×1.5 mm) were prepared (13,15) and treated with 0.4 mg/ml lysozyme in 0.05% Sarkosyl for 3 hr at room temperature. The agarose blocks were then incubated in EET containing 1 mg/ml proteinase K and 1% SDS for 20 hr at 50°C. Remaining proteinase was inactivated with 0.1 mM phenyl methyl sulfonyl fluoride (PMSF) for 1 hr, followed by several rinses in large volumes of TE buffer (1 mM EDTA, 10 mM Tris-HCl pH 8.0).

Restriction digestions and size markers

Agarose blocks with campylobacter chromosomes were put in 1 ml of restriction buffer. After 15 min. 0.9 ml buffer was removed and 50 – 100 U of restriction enzyme were added. Incubation was continued for 6 hr; another portion of enzyme was added after 3 hr.

Yeast chromosomes (Pharmacia) and a lambda ladder were used as size markers. Four μg lambda DNA (USB, Cleveland, Ohio) was ligated with 1 U of T4 ligase (Amersham) for 1 min at room temperature and directly subjected to FIGE.

FIGE

DNA samples were electrophoresed (16) at room temperature in 1% NA agarose (Pharmacia) in Tris-borate buffer (0.1 mM EDTA, 45 mM Tris-borate pH 8.3) with or without 0.5 μg/ml ethidium bromide. The FIGE program consisted of 6 to 12 identical cycles of 4 h. An exponentially increasing forward switch time from 0.1 to 55 sec. (A) or 0.1 to 10 sec. (B) was used; 50% of the switch time was reached at 40% of the cycle time. The reverse time phase and the pause time were 33% and 2% respectively of the forward time phase.

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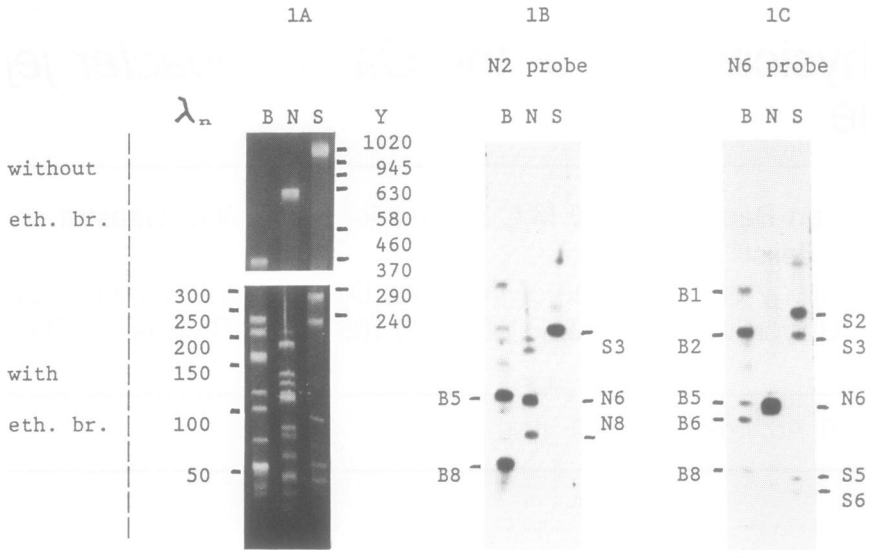

Figure 1. (A) FIGE of restriction digestions of the *C. jejuni* chromosome with *Bss*HII (lane 1), *Nci*I (lane 2) and *Sal*I (lane 3). The upper part of the photograph is of a gel run without ethidium bromide but stained afterwards and the lower part is of a gel run with ethidium bromide. Markers positions are indicated; at the right yeast chromosomes and at the left lambda concatamers. The other panels are autoradiograms of Southern hybridizations with the N6 (B) and the N2 (C) probes.

Southern blot analysis

After electrophoresis gels were UV-irradiated at 365 nm with 5.2 mW/cm^2 for 10−15 min, treated for 30 min with 0.25 M HCl, then for 1 hr with 1.5 M NaCl/0.5 M NaOH and for 1.5 hr with 1.5 M NaCl/0.5 M Tris. The DNA was transferred to nylon membrane (Hybond N, Amersham), linked to the membrane by UV irradiation, prehybridized for 3 to 4 hr at 42°C and hybridized as described (17). DNA fragments used as probe were isolated from agarose gels using glass milk (Geneclean Kit, BIO 101 Inc.). The DNA was labeled with [α-^{32}P]-dATP using random priming (Multiprimer Labeling Kit, Amersham). After hybridization, membranes were washed with 1× SSPE (17) at 42 to 55°C and exposed to an Fuji RX film. To strip probe from the blots they were washed 2 times for 30 min in 50% formamide, 0.1× SSPE, 0.1% SDS at 90°C. Blots were reused 4 to 6 times.

RESULTS

FIGE conditions

Typically, agarose blocks contained 4 to 10 μg DNA. Since the G+C content of *Campylobacter* is only 30−35% (12,15), the following restriction enzymes were tested: *Apa*I (GGGCC/C), *Bss*HII (G/CGCGC), *Eag*I (C/GGCCG), *Kpn*I (GGTAC/C), *Nae*I (GCC/GGC), *Nar*I (GG/CGCC), *Nci*I (CC/$^{G}_{C}$CG), *Sac*II (GACCT/C), *Sal*I (G/TCGAC), *Stu*I (AGG/CCT) and *Xba*I (T/CTAGA). Based on the number of generated fragments, which should be between 5 and 15, and the reproducibility of the results, *Bss*HII, *Nci*I and *Sal*I were chosen for further experiments. These three enzymes generate large (>150 kb) as well as small (<75 kb) fragments (see Table 1). Therefore, programs A and B were run sequentially to separate large and small fragments, respectively.

In Fig. 1A the upper part of the photograph is of a gel run without ethidium bromide, which showed a better resolution of large DNA fragments (>300 kb), while the lower part is of a gel run with ethidium bromide, which improved separation of smaller fragments. The N2 band appeared to be a partial digest; higher concentrations of *Nci*I made it disappear, while N6 became more intense and N8 appeared (Table 1). With conventional agarose gel electrophoresis no restriction fragments smaller than 25 kb were detected.

Table 1. Size of the *C. jejuni* chromosome; length and numbering of *Bss*HII, *Nci*I, and *Sal*I restriction fragments.

Fragment	Fragment length (kb)[1]		
	*Bss*HII	*Nci*I	*Sal*I
1	325	550	1000
2	225	[195][2]	275
3	200	170	215
4	160(a,b)	140	95
5	125	130	55
6	100	115(a,b,c)[2]	45
7	75	85	
8	55(a,b,c,d)	80[2]	
9	50	60	
10	40	45(a,b)	
11	35	35	
12		25	
total (in kb)	1715	1710	1685

[1] Fragment lengths were estimated from gels as shown in Fig. 1.
[2] The N2 fragment is a partial cleavage product, containing one of the three N6 fragments and the N8 fragment.

Estimation of the size of the *Campylobacter* chromosome

The restriction bands obtained by *Sal*I (S), *Bss*HII (B) and *Nci*I (N) digestions (Fig. 1A), were numbered as indicated in Table 1. *Bss*HII, *Nci*I and *Sal*I produced 11, 12 (of which one was a partial digestion product), and 6 bands, respectively. Some bands contained more than one fragment; based on the intensity of the bands and hybridization data we concluded that N10 and B4 consist of two fragments (a and b), N6 of three (a, b, and c) and B8 of four (a, b, c, and d). The length of all fragments was determined using lambda and yeast size markers. The average chromosome length was found to be 1,703 kb (Table 1).

Table 2. Hybridization data of *C. jejuni* DNA restriction fragments.

Probe	Signal intensity					
	High			Low		
	*Bss*HII	*Nci*I	*Sal*I	*Bss*HII	*Nci*I	*Sal*I
N1	B3 B4	N1 S1	B2 B8 B9 B10	–	–	
N2	B5 B8	N6 N8	S3	–	–	–
N3	B1 B8	N3	S1	–	–	–
N4	B7 B8(2×)[2]	N4	S1	–	–	–
N5	B6 B11 B4	N5	S2	–	–	–
N6[3]	B2	N6(3×)	S2 S3	B1 B5 B6	–	S5 S6
N7	B1	N7	–	–	–	S1
N8	B8	N8	–	–	–	–
N9	B8	N9	S4	B4	–	–
N10	B4	N10	–	B1 B8	–	–
N11	B8	N11	NT[1]	–	–	NT
N12	B4	N12	S2	–	–	–
B1	B1	N3	S1	–	N6 N7 N10	–
B2	B2	N1	N6(2×)	S5 S6 S1	–	–
B3	B3	N1	–	–	–	S1
B4	B4(2×)	N1 N5 N9 N10 N12	S1 S2 S4	–	–	–
B5	B5	N2	N6(2×)	S3	–	–
B6	B6	N5 N6	S2	–	–	–
B7	B7	N4	S1	–	–	–
B8	B8(3×)	N2 N4 N9	S1	–	N1 N3 N11	S3 S4
B9	B9	N1	S1	–	–	–
B10	B10	N1	S1	–	–	–
B11	B11	N5	S2	–	–	–
S3	B5 B8	N2 N6	S3	B2	N1 N11	–
S4	B4 B8	N9 N10	S4	–	–	–
S5	B2	N6	S5	–	–	–
S6	B2	N6	S6	–	–	–

[1] NT; not tested
[2] indicates double or triple signal strenght
[3] probes containing multiple fragments are underlined

Physical map of the *C. jejuni* chromosome

The physical map is based on hybridization studies. All bands, as shown in Fig. 1A, were isolated from gels and used as probes in Southern blot experiments. The data of the hybridization experiments are summarized in Table 2. Two examples, in which N6 and N2 were used as probes, are shown in Fig. 1B and 1C, respectively. Theoretically, the signal strength is directly related to the overlap of a fragment with the probe; of which the latter consists of a mixture of labeled, single stranded DNA molecules all shorter than the isolated fragment of the chromosome. Therefore, the occurence of weak signals can be used as an indication for a partial overlap of the weakly reacting band with the probe.

The construction of the map was started with the ordering of the following fragments; B2 hybridized with S1, S5, and S6 and with a lower intensity with S3. In addtion, B2 reacted with N1 which is within S1, with the N6 fragment covering S5 and S6 and with a second N6 fragment. The reasoning that S2 is next to S1 comes from the hybridization of the third, yet unassigned N6 fragment with B1 and B6. B1 hybridizes with S1 and B6 with S2. Other fragments could be ordered using the data in Table 2. The resulting physical map is shown in Fig. 2. The map shows that the genome has to be circular, which was confirmed by the absence of a major 1700 kb band after FIGE of undigested chromomal DNA (data not shown). We chose the center of fragment N4 as the zero position. The relative position of a few fragments could not be determined e.g. the N7/N10, S5/S6, B5/B8, and B3/B4/B9/B10 fragments. The order of the B3, B4,

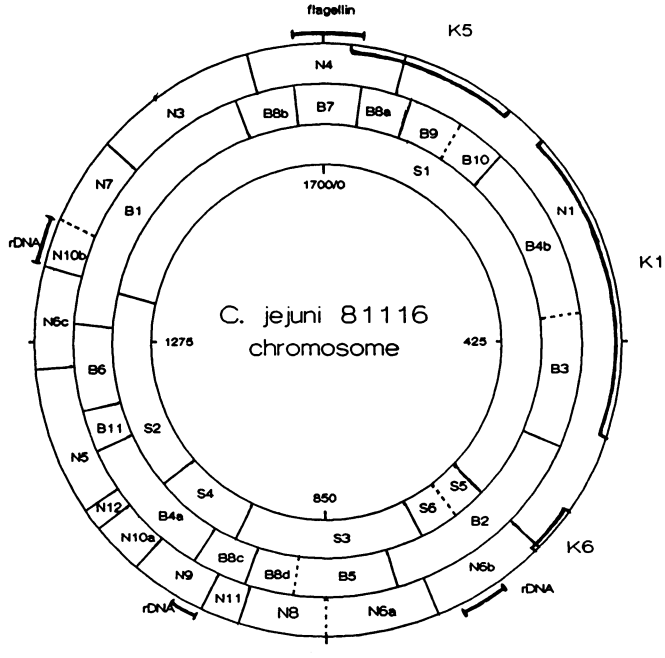

Figure 2. Physical map of the *C. jejuni* strain 81116 chromosome. The location of the restriction fragments was determined using the data from Table 2; in the inner circle the map positions are indicated corresponding to the length of the chromosome. The middle of B7 was choosen as the zero position. The location of flagellin and rRNA genes are derived from Fig. 3.

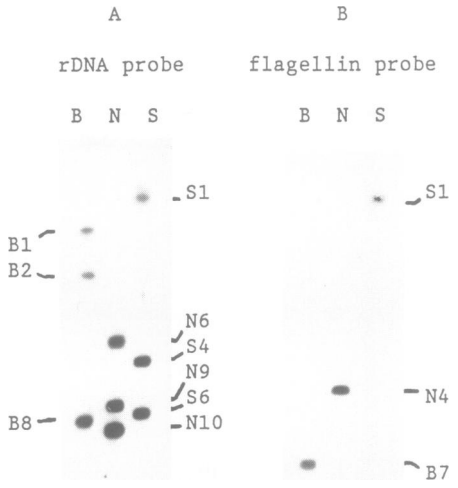

Figure 3. Southern blot analysis to determine the location of flagellin (A) and rRNA genes (B). Hybridizing bands are indicated.

B9 and B10 fragments was further investigated. *Kpn*I digestion of the chromosome produced at least 8 fragments, of which K1, K5 and K6 reacted with the N1 probe. Southern blots indicated the location of these fragments as shown in Fig. 2.

Localization of flagellin and ribosomal RNA genes

We mapped the conserved rRNA loci and the genes encoding flagellin on the *C. jejuni* chromosome. The Southern blot results are shown in Fig. 3A and 3B. The flagellin genes were mapped around position 0, and the rDNA loci at position 650, 900 and 1,300 (Fig. 2).

DISCUSSION

The size of the *C. jejuni* chromosome determined with FIGE differed from the earlier reported size of the campylobacter chromosome, but agrees with recent data of Chang and Taylor (personal communication). The restriction sites for the enzymes used in construction of the map are not equally distributed on the chromosome; the region between position 200 and 600 contains only few restriction sites. A similar situation exists in the chromosomes of *Bacillus cereus* (18), *Escherichia coli* (19), *Haemophilus influenzae* (20), *H. parainluenzae* (21), *Mycoplasma mycoides* (22), *M. genitalium* (23), *Thermococcus celer* (24) and *Ureaplasma urealyticum* (25).

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