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Genome Analysis of *Bacteroides* by Pulsed-field Gel Electrophoresis: Chromosome Sizes and Restriction Patterns

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Abstract

The chromosomal DNAs of nine strains of seven *Bacteroides* species including *B. fragilis*, the type species of the genus *Bacteroides*, were digested with rare-cutting restriction enzymes I-*Ceu* I, *Not* I, and *Asc* I and analysed by pulsed-field gel electrophoresis. The genome sizes of *B. fragilis*, *B. distasonis*, *B. eggerthii*, *B. ovatus*, *B. thetaiotaomicron*, *B. uniformis*, and *B. vulgatus* were determined to be 5.3, 4.8, 4.4, 6.9, 4.8, 4.6, and 5.1 Mbp, respectively. *B. distasonis* and *B. vulgatus*, and also *B. uniformis* and *B. eggerthii*, showed simillar I-*Ceu* I restriction profiles. I-*Ceu* I cut *B. uniformis* and *B. eggerthii* genomes into four, *B. ovatus* into five, *B. fragilis* and *B. thetaiotaomicron* into six, and *B. distasonis* and *B. vulgatus* into seven fragments. On the basis of genome size, restriction profile, and I-*Ceu* I fragment number, a phylogenetic tree of the *Bacteroides* species was proposed. This was in overall agreement with the previous phylogenetic tree obtained by 16S rRNA data, with the exceptions of *B. distasonis* and *B. ovatus*.

Key words: *Bacteroides* species; PFGE; genome size; restriction pattern; phylogenetic relationship

1. Introduction

Gram-negative anaerobic bacteria are often opportunistic pathogens that can be isolated from many types of infections.[1,2] The anaerobes most frequently isolated from human clinical specimens are members of the genus *Bacteroides*. One species, *B. fragilis*, accounts for over half of these isolates. *Bacteroides* species formerly referred to as the "*B. fragilis* group,"[3] such as *B. fragilis*, *B. thetaiotaomicron*, *B. distasonis*, *B. ovatus*, *B. uniformis*, *B. vulgatus*, and *B. eggerthii*, were recently proposed as *Bacteroides* sensu stricto,[4–7] consisting of ten saccharolytic nonpigmenting species.

The standard method for identification and classification of anaerobic bacteria uses carbohydrate fermentation and other biochemical tests in combination with metabolic end-product analysis by gas chromatography.[3] In some cases, however, it is difficult to differentiate *Bacteroides* species because they behave similarly to each other on many biochemical tests.[3,8] Despite this, they are not closely related; DNA-DNA hybridization studies indicate that none of the *Bacteroides* species shared more than 30% DNA homology with any other species.[9] Therefore, characterization of their global genome organization may provide a good approach for accurate differentiation of these species. Pulsed-field gel electrophoresis (PFGE) is a powerful technique for studying the size and organization of bacterial genomes.[10–12] Identification of species has been attempted with the use of rare-cutting restriction endonucleases followed by separation of the fragements of PFGE. The procedure has been applied successfully to *Pseudomonas* species.[13]

In the present study we determined the chromosome sizes of seven *Bacteroides* species by summing the sizes of DNA fragments obtained by digestion with three different rare-cutting enzymes. We also compared their restriction patterns to gain a better understanding of phylogenetic relationships among the species.

2. Materials and Methods

2.1. Bacterial strains and culture conditions

Bacterial strains used in this study are *B. fragilis* ATCC25285, *B. fragilis* YCH46,[14–16] *B. thetaiotaomicron* ATCC29148, *B. ovatus* ATCC8483, *B. eggerthii* ATCC27754, *B. distasonis* ATCC8503, *B. distasonis* Takeda 1621, *B. uniformis* ATCC8492, and *B. vulgatus*

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Werner F-92. Bacteria were grown anaerobically in 15 ml of GAM broth (Nissui Pharmaceutical Co., Tokyo) at 37°C for 36–48 h to an A_{600} of 0.8–1.0.

2.2. *Preparation of DNA in agarose blocks*

Cells were harvested from 1 ml culture by centrifugation, washed, and resuspended in 0.5 ml of 0.05 M EDTA (pH 8.0). The cell suspension was mixed with an equal volume of 1% (w/v) low-melting-point agarose in 0.125 M EDTA which had been kept at 50°C and pipetted into block molds (Bio-Rad, Richmond, Calif.) to form agarose gel blocks (250 μl/block). The agarose blocks were transferred to 20 ml of lysis solution containing 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 100 mM EDTA, 0.5% sarkosyl, 20 μg/ml RNase, and 1 mg/ml lysozyme and incubated overnight at 37°C. The gel blocks were transferred to 15 ml of deproteinized solution containing 100 mM EDTA, 0.5% sarkosyl, 1 mg/ml proteinase K and incubated at 37°C for 48 h. The blocks were washed in 15 ml of storage solution (10 mM Tris-HCl, pH 8.0; 100 mM EDTA) and treated with PMSF solution (1 mM phenylmethylsulfonyl fluoride in the storage solution) for 1 h at room temperature. After two washes in storage solution, the gel blocks were stored at 4°C.

2.3. *Restriction endonuclease digestion of DNA in agarose blocks*

The restriction enzymes used were obtained from New England Biolabs. One-third portion of an agarose gel block was used for each digestion. Prior to restriction digestion, the gel piece was washed twice in 3 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) for 30 min at room temperature and soaked for 30 min at room temperature in 250 μl of reaction buffer. Then the buffer was replaced with 250 μl of complete reaction mixture containing a restriction enzyme, and the mixture was incubated at 37°C for 8 to 16 h. *Asc* I, I-*Ceu* I, and *Not* I digestions were performed with 10 U, 2 U, and 20 U of the enzymes, respectively. Double digestions were performed by repeating the procedure described above with the second enzyme.

2.4. *Pulsed-field gel electrophoresis*

Contour-clamped homogeneous electric field electrophoresis was performed with a Bio-Rad CHEF-DRII system. DNA fragments were separated into 1% (unless otherwise noted) agarose gel in 0.5×TBE buffer at 14°C. Different electrophoresis conditions were used for better resolution of the restriction fragments (see Figure legends). Four molecular size markers were used: *Hansenula wingei* chromosomal PFGE marker (Bio-Rad), *Saccharomyces cerevisiae* chromosomal PFGE marker, lambda ladder PFGE marker, mid range PFGE marker I (all from New England Biolabs). DNA bands were visualized by staining with ethidium bromide and exposure to UV illumination. Densitometric tracings of photographs of each gel were used to determine the number of co-migrating fragments which could not be resolved. Fragment sizes were determined by comparison with the size markers. Bands were sized only in the linear portion of the calibration curve. The average of at least three separate analysis was used to determine fragment sizes.

3. Results

3.1. *Selection of restriction enzymes*

The first step in characterizing the *Bacteroides* genome was to identify restriction enzymes which digest the genomic DNA into a small number of fragments. Since the organisms of *Bacteroides* species have relatively narrow G+C range (ca. 40 to 48 mol%),[17,18] several rare-cutting restriction enzymes with GC-rich recognition sites were tested. The best results were obtained with *Not* I and *Asc* I; they produced 14 and 4 bands, respectively, from the *B. fragilis* YCH46 genome (lane f, Fig. 1B and C). *Sfi* I seemed to be less useful for sizing the genome because this enzyme generated more than 25 bands containing several doublets or even triplets which were difficult to resolve (data not shown). Other enzymes tested, such as *Bln* I, *Cpo* I, *Sma* I, and *Sse* I, yielded too many small-sized fragments. The enzyme I-*Ceu* I that cleaves a specific 19-bp sequence in the *rrl* gene (23S rRNA) of many eubacteria[19–21] was also useful; this enzyme produced four to seven bands from the strains tested (Fig. 1A). Thus, I-*Ceu* I, *Not* I, and *Asc* I, were used for the subsequent analysis of genome sizes and restriction patterns of *Bacteroides* strains.

3.2. *Estimation of genome sizes*

Typical PFGE patterns of single digests of DNAs from seven *Bacteroides* species with I-*Ceu* I, *Not* I, or *Asc* I are shown in Fig. 1. Intense bands that appeared to be comprised of two or more co-migrating fragments were resolved into individual fragments by varying the pulse conditions. For example, the first band from the bottom in lane d of Fig. 1A could be separated into two closely spaced fragments of 160 and 152 kb with a 5–25 s ramping pulse at 150 V for 48 h (data not shown). Under the same conditions, a 72-kb fragment which had been run out of gel in lane v of Fig. 1A was detected. Other doublets and triplets were separated in the same way. Thus, the number of the restriction fragments produced from each *Bacteroides* strains was determined as summarized in Table 2. All the *Bacteroides* strains yielded DNA fragments with sizes larger than 1000 kb, which may give inaccurate values for a total genome size. In such cases,

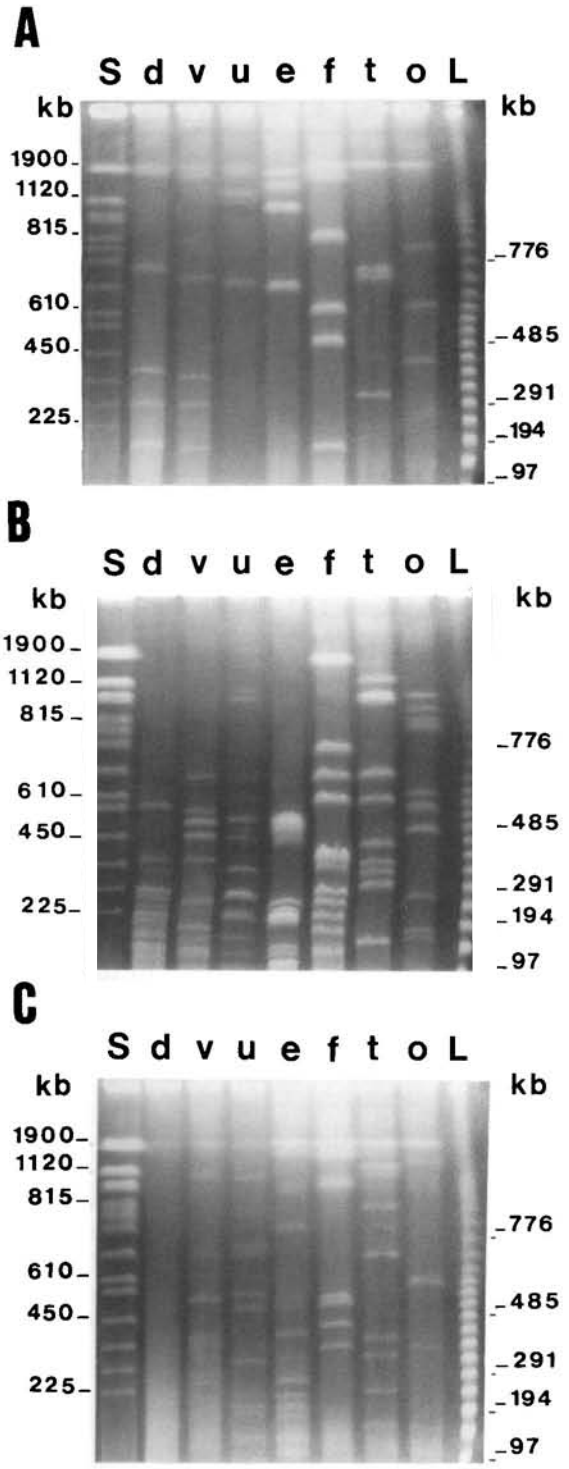

Figure 1. Restriction patterns of *Bacteroides* genomes with I-*Ceu* I (A), *Not* I (B), and *Asc* I (C). Lanes: d, *Bacteroides distasonis* ATCC8530; v, *B. vulgatus* Werner F-92; u, *B. uniformis* ATCC8492; e, *B. eggerthii* ATCC27754; f, *B. fragillis* YCH46; t, *B. thetaiotaomicron* ATCC29148; o, *B. ovatus* ATCC8483; S, Yeast chromosome PFGE marker (*Saccharomyces cerevisiae*); and L, Lambda ladder PFGE marker. The following pulse conditions were used: pulses 70 s for 15 h and 120 s for 10 h at 200 V, followed by pulse times ramped from 5 to 120 s for 15 h at 150 V.

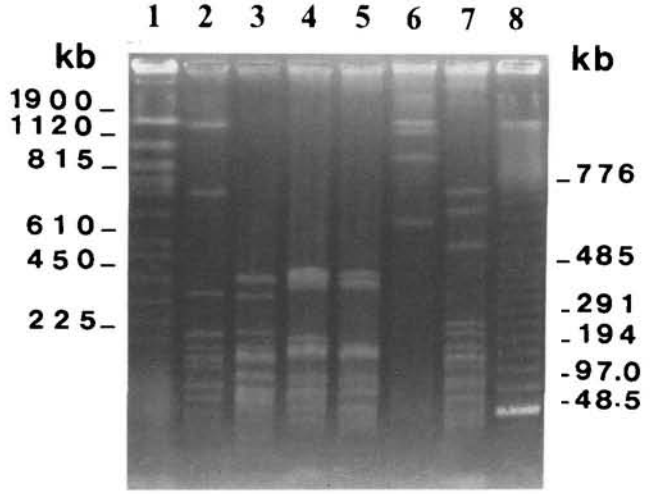

Figure 2. Fragments of the *B. eggerthii* ATCC27754 genome obtained by digestions with *Asc* I (Lane 2), *Asc* I+*Not* I (Lane 3), *Not* I (Lane 4), *Not* I+I-*Ceu* I (Lane 5), I-*Ceu* I (Lane 6), and *Asc* I+I-*Ceu* I (Lane 7). Lane 1 contains Yeast chromosome PFGE marker; and Lane 8, Lambda ladder PFGE marker. The gel was run at 200 V with pulse times of 70 s for 15 h and 120 s for 10 h, followed by a linear pulse ramp of 5–100 s for 12 h at 100 V.

double digestions in various combinations of three enzymes were performed to cleave the larger or co-migrating fragments into smaller fragments. An example of single and double digestions for *B. eggerthii* ATCC27754 is shown in Fig. 2. PFGE were also performed under different conditions to obtain more accurate sizes of larger and smaller fragments (Fig. 3). The sizes of the fragments resolved in this way were determined by comparison with known molecular size markers. The results for *B. eggerthii* ATCC27754, for example, are summarized in Table 1 with the genome size estimated by summing the sizes of single- or double-digestion fragments. Gel electrophoresis with no pulse (constant-field gel electrophoresis) showed that none of the digestions generated fragments smaller than those listed (data not shown). The genome sizes estimated in a similar manner for the *Bacteroides* strains tested are listed in Table 2. The most reliable genome size for each strain was taken from the digestion where it contained no fragments larger than 1000 kb and had the smallest number of co-migrating fragments.

Large circular molecules such as intact chromosomes do not migrate into PFGE unless they are smaller than 200 kb in size. All the undigested DNAs from the *Bacteroides* strains remained at the gel origin, even when electrophoresis was performed at lower voltages and longer pulse times (data not shown). This observation suggests that, similar to the majority of bacteria, the *Bacteroides* chromosomes are circular in nature. No

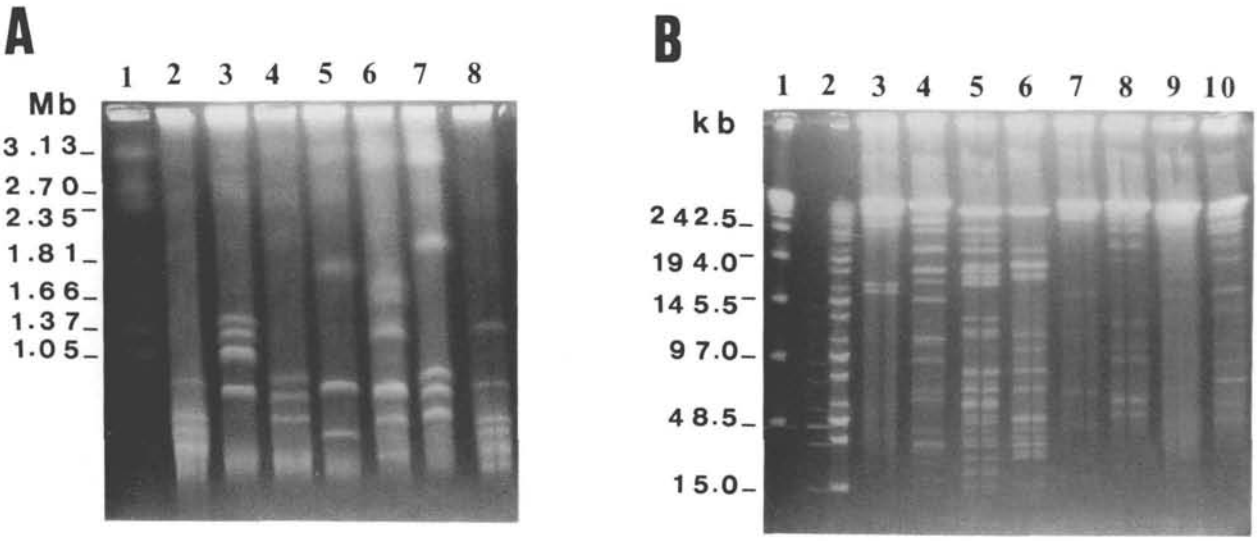

Figure 3. Resoultion of larger and smaller restriction fragments. (A) Larger fragments ranging 1.05–3.13 Mb were resolved. Genomic DNAs of *Bacteroides distasonis* Takeda 1621 (Lane 2), *B. eggerthii* ATCC27754 (Lane 3), *B. ovatus* ATCC8483 (Lane 4), *B. thetaiotaomicron* ATCC29148 (Lane 5), *B. ovatus* ATCC29148 (Lane 7), and *B. vulgatus* Werner F-92 (Lane 8) were digested with I-*Ceu* I, and the genomic DNA of *B. ovatus* ATCC29148 (Lane 6) digested with *Asc* I. PFGE was performed in 0.8% agarose gel at 100 V with a 500 s switching pulse for 48 h, followed by a second electrophoresis at 150 V with a 500 s switching pulse for 24 h. Lane 1, CHEF DNA size markers *Hansenula wingei* YB-4662-VIA chromosomes. (B) Smaller fragments ranging 15–150 kb were seperated by a linear pulse ramp of 5 to 25 s at 150 V for 40 h. Genomic DNAs of *Bacteroides distasonis* Takeda 1621 (Lane 3), *B. eggerthii* ATCC27754 (Lane 4), *B. ovatus* ATCC8483 (Lane 7 and Lane 9), *B. thetaiotaomicron* ATCC29148 (Lane 8), and *B. vulgatus* Werner F-92 (Lane10) were digested with *Asc* I+I-*Ceu* I. The genomic DNA of *B. eggerthii* ATCC27754 was digested with *Asc* I+*Not* I (Lane 5) and *Not* I+I-*Ceu* I (Lane 6). Lane 1 contains Lambda ladder PFGE marker; and Lane 2, Lambda ladder mid-range PFGE marker I.

smaller molecules, suggestive of extrachromosomal elements, were detected in the strains tested.

3.3. Analysis of restriction patterns

It was of interest to study whether more closely related species within the genus *Bacteroides* showed more similarities in the restriction pattern. In general, the restriction patterns were distinct for each species (Fig. 1); on visual comparison, only a few common bands were found in certain strains of the seven species. Nevertheless, some trends can be noted. In particular, it is worth noting that I-*Ceu* I profiles of *B. distasonis* and *B. vulgatus* are very similar (lanes d and v, Fig. 1A). *B. eggerthii* and *B. uniformis* also showed similar I-*Ceu* I patterns (lanes e and u, Fig. 1A). The I-*Ceu* I fragments of 650–700 kb in size are present in *B. distasonis*, *B. vulgatus*, *B. eggerthii*, and *B. uniformis*. These four species are isolated less frequently from clinical specimens. Most of the *Not* I fragments produced from the four species were less than 600 kb in sizes (lanes d, v, e, and u in Fig. 1B). This is in contrast to *B. fragilis*, *B. thetaiotaomicron*, and *B. ovatus* (lanes f, t, and o in Fig. 1B) that produced several *Not* I fragments larger than 600 kb. The former two of the three are isolated with greater frequencies. It should also be noted that two *Not* I fragments with sizes of 610 and 660 kb are in common between *B. fragilis* and *B. thetaiotaomicron*. In the *Asc* I profiles of the *Bacteroides* strains, no significant similarity was found. However, it was striking that *B. distasonis* Takeda 1621 appeared resistant to the action of *Asc* I, unlike other *Bacteroides* strains that produced 7 to 18 *Asc* I fragments from their genomes. Only a faint slow-migrating band was detected in the lane (lane d, Fig. 1C). This band may not represent a single species of DNA but sheared DNA because the band was diffuse when PFGE was performed under conditions in which larger linear fragments were resolved (data not shown). Moreover, restriction patterns of *Not* I single and *Not* I-*Asc* I double digestions of the DNA were found to be identical. The I-*Ceu* I and I-*Ceu* I-*Asc* I profiles were also identical. These results suggest the absence of a *Asc* I site in the *B. distasonis* Takeda 1621 genome. DNA from another strain, *B. distasonis* ATCC27754, was also resistant to *Asc* I and showed identical *Not* I and I-*Ceu* I patterns to those of *B. distasonis* Takeda 1621. *B. fragilis* is considered as clinically most important species because it is the most frequently isolated species from various sites of infection. To see whether PFGE analysis can be used to distinguish within this species, restriction patterns of two *B. fragilis* strains, ATCC25285 and YCH46, were compared. Similarities are apparent between the two strains

Table 1. Sizes of fragments of the *B. eggerthii* chromosome produced by restriction enzymes *Asc* I, I-*Ceu* I and *Not* I.

Fragment size (kb) obtained with					
Asc I	*Asc* I+*Not* I	*Not* I	I-*Ceu* I+*Not* I	I-*Ceu* I	*Asc* I+I-*Ceu* I
1473	454	524(2)	524	1538	760
760	382	488(2)	489(2)	1333	706
421	265	432(2)	430(2)	981	607
268	214(2)	247	410	675	296
255	184(2)	229	229		255
241	176(2)	175(2)	175(2)		210(2)
210	163(2)	160	160(2)		180(2)
180	156(2)	119	117		172
168	128(2)	103	99		155
155	120(2)	71	72		121
115	117(2)	61	64		115
72	107	52	52(2)		92
64	87(2)	35	41		72
41	80	28	35		64
32	75(2)	17	32		41
28	68(2)		28		32(2)
25	55		22		28
18	51		17		25
	43				18
	35				
	32				
	25				
	22				
	20				
	16				
	15				
4526	4578	4360	4302	4527	4371

The number in parenthesis indicates the number of co-migrating fragments.
The most reliable value (see Results) is underlined.

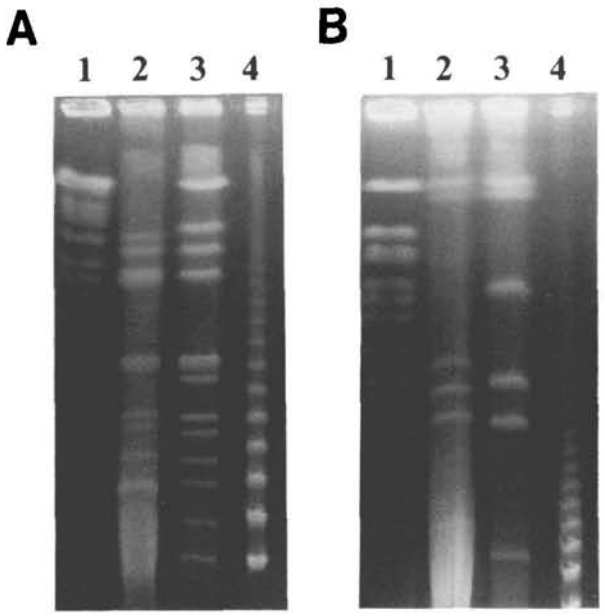

Figure 4. Comparison of PFGE patterns of *B. fragilis* strains ATCC25285 and YCH46. *Not* I digestion of ATCC25285 (lane 2) and YCH46 (lane 3). I-*Ceu* I digestion of YCH46 (lane 2) and ATCC25285 (lane 3). Lanes: 1, Yeast chromosome PFGE marker; 2, Lambda ladder PFGE marker. PFGE conditions were the same as performed in Fig. 1.

in their *Not* I (Fig. 4A) and I-*Ceu* I (Fig. 4B) profiles, but the strains could easily be distinguished from each other. The genome sizes of the two *B. fragilis* strains are very close to each other (Table 2). Although we tested only two strains, we anticipate that PFGE can be used for epidemiological studies to determine the degree of relatedness among different strains of this species.

4. Discussion

The taxonomy of the genus *Bacteroides* has been considered unsatisfactory and revised several times in the past few years.[4–7] The application of biochemical, chemical, and more recently, molecular biological techniques has done much to clarify the intergenic and intragenic structure of *Bacteroides*. On the basis of these data, Shah and Collins[6] proposed that the genus should be restricted to *Bacteroides* sensu stricto: the type species, *B. fragilis*, and closely related species. However, species of *Bacteroides* still appear in need of reclassification. Recently, Paster et al.[22] proposed that *B. distasonis* should be placed within the *Porphyromonas* cluster on the basis of 16S rRNA sequence analysis, which has currently been done for classification of many bacteria. However, as suggested by Fox et al.,[23] 16S rRNA sequence is not necessarily a sufficient criterion for defining species. Other criteria complementing the 16S rRNA data must be used. One such criterion may be established by genome analysis, including estimation of genome sizes, comparison of restriction profiles, and determination of the number of *rrn* loci.

One of the major aims of this study was to determine the genome sizes for the *Bacteroides* species. As might be expected, the genome sizes are well conserved within closely related bacteria; for instance, the chromosome sizes of *Escherichia coli*, *Shigella flexneri*, and *Salmonella typhimurium* are 4.6, 4.6 and 4.8 Mb respectively.[11] However, genome size may vary drastically within a single taxonomic group (1 to 5 Mb for *Spirochaeta* spp; 2.7 to 6.5 Mb for cyanobacteria).[12] The *Bacteroides* strains had a relatively narrow genome-size range (from 4.4 Mb for *B. eggerthii* ATCC27754 to 6.9 Mb for *B. ovatus* ATCC8483). These sizes are positioned midway in the scale of bacterial genome sizes, which range from 0.6 Mb for *Mycoplasma genitalium* to 9.5 Mb for *Myxococcus xanthus*.[12]

The restriction enzyme I-*Ceu* I cleaves a specific 19-bp sequence which is highly conserved in the *rrl* gene (23S rRNA) of many eubacteria and absent from the rest of the genome.[12] Therefore, the number of fragments obtained by macrorestriction analysis using I-*Ceu* I is indicative

Table 2. Genome sizes of Bacteroides strains determined after PFGE.

Strain	Size obtained by restriction enzyme digestion (kb)						Average size (kb)
	Asc I	*Asc* I + *Not* I	*Not* I	I-*Ceu*-I + *Not* I	I-*Ceu*I	*Asc* I + I-*Ceu*I	
B. fragilis ATCC25285	5203 (6)	ND	5219 (19)	ND	5168 (6)	ND	5196
B. fragilis YCH46	5270 (7)	5295 (24)	5188 (17)	ND	5269 (6)	ND	5252
B. eggerthii ATCC27754	4526 (18)	4587 (37)	4360 (19)	4302 (23)	4527 (4)	4371 (22)	4401
B. distasonis ATCC8503	ND (0)	4800 (25)	4800 (25)	ND	4835 (7)	4835 (7)	4817
B. distasonis Takeda1621	ND (0)	4800 (25)	4800 (25)	ND	4835 (7)	4835 (7)	4817
B. ovatus ATCC8483	6696 (7)	6885 (19)	6568 (12)	6642 (17)	6812 (5)	6445 (12)	6674
B. thetaiotaomicron ATCC29148	4752 (8)	4657 (22)	4812 (14)	4757 (20)	4431 (6)	4752 (14)	4693
B. uniformis ATCC8492	4676 (13)	4886 (30)	4955 (17)	4718 (21)	4977 (4)	4611 (17)	4804
B. vulgatus Werner F-92	5030 (12)	5373 (31)	5117 (19)	5016 (26)	5262 (7)	5184 (19)	5171

The number in parenthesis indicates the number of restriction fragments generated by the respective enzyme(s) used.
The most reliable value for each genome size (see Results) is underlined.
ND: not determined.

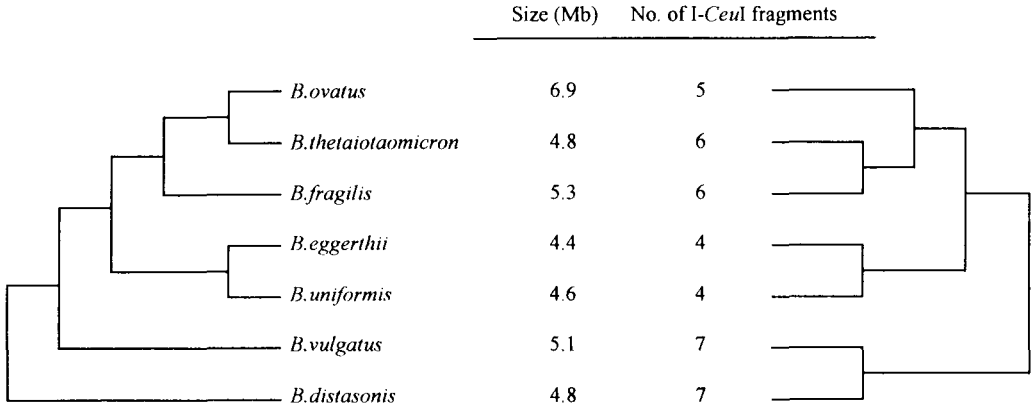

Figure 5. The phylogenetic tree proposed by Paster et al.,[22] based on 16S rRNA sequence similarities (left) and the phylogenetic tree proposed here based on the number of I-*Ceu* I fragments and I-*Ceu* I digestion pattern similarities (right).

of *rrl* gene number in the genome. This enzyme cuts the chromosome of *E. coli* and *Salmonella* spp. into seven fragments[21] and *Clostridium perfringens*[24] and *Bacillus subtilis* into ten.[25] From these data, it might be expected that closely related bacteria have the same number of I-*Ceu* I segments in their genomes. However, the number of I-*Ceu* I fragments varied within the genus *Bacteroides*, varying from four to seven. This allowed us to classify the seven *Bacteroides* species into four classes: class I with four I-*Ceu* I fragments includes *B. uniformis* and *B. eggerthii*; class II with five fragments, *B. ovatus*; class III with six, *B. fragilis* and *B. thetaiotaomicron*; and class IV with seven, *B. distasonis* and *B. vulgatus*. It is worth noting that genome sizes of the species in each class are well conserved. On the basis of the genome size, restriction pattern, and number of I-*Ceu* I fragments, we propose here a phylogenetic tree of the seven *Bacteroides* species (Fig. 5).

Phylogenetic relationships of the *Bacteroides* species determined in this study were, with few exceptions, in overall agreement with those obtained by Paster et al.[22] from 16S rRNA sequence data. One exception was their proposal that *B. distasonis* should be included within the genus *Porphyromonas*. A unique feature of *B. distasonis* that its genomic DNA is resistant to *Asc* I appears to be consistent with the notion. However, in a review of their data, *B. distasonis* appeared equally distant from the species of *Bacteroides* and those of *Porphyromonas*, or even more closely related to the *Bacteroides* species than those of *Porphyromonas* (average similarity of 85.6% and 83.5%, respectively). From the PFGE data presented here, *B. distasonis* should form a cluster with *B. vulgatus* (Fig. 5), although these two species share only 84.2% 16S rRNA sequence similarity.[22] Because of this phylogenetic depth, it is still uncertain whether *B. distasonis* and *B. vulgatus* should be considered as species of *Bacteroides* or *Porphyromonas*, or whether they constitute one or more separate genera. Further studies, including genome analysis of *Porphyromonas* species, are needed to clarify the taxonomic status of these two species. Another exception is the phylogenetic position of *B. ovatus*. The ATCC strain of this species is distinct from others for its larger genome size and its number of I-*Ceu* I fragments. *B. fragilis* and *B. thetaiotaomicron*, both having 6 I-*Ceu* I fragments and similar genome sizes, seems to be more closely related. *B. thetaiotaomicron* should form

a cluster not with *B. ovatus* but with *B. fragilis*, accordingly. It is of interest that, in a recent clinical survey, *B. thetaiotaomicron* is the species isolated with the second greatest frequency next to *B. fragilis*.[26]

The results presented here may provide a basis for future studies, such as construction of physical and genetic maps, to extend our understanding of the phylogeny of the genus *Bacteroides*. To our knowledge, this is the first report of the restriction patterns and genome sizes of *Bacteroides* species using PFGE. This technique is useful to study the phylogenetic relationships among species belonging to the same genus.

References

1. Finegold, S. M. and George, W. L. 1989, Anaerobic infections in humans, Academic Press, Inc., San Diego, California
2. Jousimies-Somer, H. R. and Finegold, S. M. 1991, Anaerobic gram-negative bacilli and cocci., In: Balows, A., Hausler, W. J. Jr., Herrmann, K. L., Insenberg, H. D., and Shadomy, H. J. (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D. C., p. 538–553.
3. Holdeman, L. V., Cato, E. P., and Moore, W. E. C. (ed). 1977. Anaerobic laboratory manual, 4th ed., VPI Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.
4. Collins, M. D. and Shah, H. N. 1987, Recent advances in the taxonomy of the genus *Bacteroides*, p. 249–258. In: Borriello, S. P., Hardie, J. M., Drasar, B. S., Duerden, B. I., Hudson, M. J., and Lysons, R. J. (ed.), Recent advances in anaerobic bacteriology, Martinus Nijhoff Publishers, Dordrecht, The Netherland.
5. Shah, N. H. and Collins, M. D. 1988, Proposal for reclassification *of Bacteroides asaccharolyticus*, *Bacteriodes gingivalis*, and *Bacteroides endodentalis* in a new genus *Porphyromonas*, *Int. J. Syst. Bacteriol.*, **38**, 128–131.
6. Shah, H. N. and Collins, M. D. 1989, Proposal to restrict the genus *Bacteroides* (Castellani and Chalmers) to *Bacteroides fragilis* and closely related species, *Int. J. Syst. Bacteriol.*, **39**, 85–87.
7. Shah, H. N. and Collins, M. D. 1990, *Prevotella*, a new genus to include *Bacteroides melaninogenicus* and related species formerly classified in the genus *Bacteroides*, *Int. J. Syst. Bacteriol.*, **40**, 205–208.
8. Shah, H. N. and Collins, M. D. 1983, Genus *Bacteroides*: a chemotaxonomical perspective, *J. Appl. Bacteriol.*, **55**, 403–416.
9. Robert, M. C., Moncla, B., and Kenny, G. E. 1987, Chromosomal DNA probes for the identification of *Bacteroides* species, *J. Gen. Microbiol.*, **133**, 1423–1430.
10. Krawiec, S. and Riley, M. 1990, Organization of the bacterial chromosome, *Microbiol. Rev.*, **54**, 502–539.
11. Cole, S. T. and Girons, I. S. 1994, Bacterial genomics, *FEMS Microbiol. Rev.*, **14**, 139–160.
12. Fonstein, M. and Haselkorn, R. 1995, Physical mapping of bacterial genomes, *J. Bacteriol.*, **177**, 3361–3369.
13. Grothues, D. and Tümmler, B. 1991, New approaches in genome analysis by pulsed-field gel electrophoresis: application to the analysis of *Pseudomonas* species, *Mol. Microbiol.*, **5**, 2763–2776.
14. Ono, T., Akimoto, S., Kinouchi, T., Kataoka, K., and Ohnishi, Y. 1994, Cloning and expression of the *Bacteroides fragilis* YCH46 neuraminidase gene in *Escherichia coli* and *Bacteroides uniformis*, *FEMS Microbiol. Lett.*, **121**, 153–158.
15. Akimoto, S., Ono, T., Tsutsui, H., Kinouchi, T., Kataoka, K., and Ohnishi, Y. 1994, Complete sequence of the *Bacteroides fragilis* YCH46 neuraminidase-encoding gene, *Biochem. Biophys. Res. Commun.*, **203**, 914–921.
16. Chen, Y. C., Kinouchi, T., Kataoka, K., Akimoto, S., and Ohnishi, Y. 1995, Purification and characterization of a fibrinogen-degrading protease in *Bacteroides fragilis* strain YCH46, *Microbiol. Immunol.*, **39**, 967–977.
17. Johnson, J. L. 1978, Taxonomy of *Bacteroides*. I. Deoxyribonucleic acid homologies among *Bacteroides fragilis* and other saccharolytic *Bacteroides* species, *Int. J. Syst. Bacteriol.*, **28**, 45–256.
18. Holdeman, L. V., Kelly, R. W., and Moore, W. E. C. 1984, Genus I. *Bacteroides* Castellani and Chalmers 1919, 959, 604–631. In Krieg, N. R. and Holt, J. G. (ed), Bergey's manual of systematic bacteriology, 1st ed. The Williams and Wilkins Co., Baltimore.
19. Liu, S.-L., Hessel, A., and Sanderson, K. E. 1993a, Genomic mapping with intron-encoded I-*Ceu* I, an intron-encoded endonuclease, specific for genes for ribosomal RNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria, *Proc. Natl. Acad. Sci. USA*, **90**, 6874–6878.
20. Liu, S.-L., Hessel, A., and Sanderson, K. E. 1993b, The *Xba* I-*Bln* I-*Ceu* I genomic cleavage map of *Salmonella typhimurium* LT2 determined by double digestion, end-labelling, and pulsed-field gel electrophoresis, *J. Bacteriol.*, **175**, 4104–4120.
21. Liu, S.-L., and Sanderson, K. E. 1995, I-*Ceu* I reveals conservation of the genome of independent strains of *Salmonella typhimurium*, *J. Bacteriol.*, **177**, 3355–3357.
22. Paster, B. J., Dewhirst, F. E., Olsen, I., and Fraser, G. J. 1994, Phylogeny of *Bacteroides*, *Prevotella* and *Porphyromonas* spp. and related bacteria, *J. Bacteriol.*, **176**, 725–732.
23. Fox, G. E., Wisotzkey, J. D., and P. Jurtshuk, Jr. 1992, How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity, *Int. J. Syst. Bacteriol.*, **42**, 166–170.
24. Katayama, S., Dupuy, B., Garnier, T., and Cole, S. T. 1995, Rapid expansion of the physical and genetic map of the chromosome of *Clostridium perfringens* CPN50, *J. Bacteriol.*, **177**, 5680–5685.
25. Toda, T. and Itaya, M. 1995, I-*Ceu* I recognition sites in the *rrn* operons of the *Bacillus subtilis* 168 chromosome: inherent landmarks for genome analysis, *Microbiol.*, **141**, 1937–1945.
26. Brook, I. 1995, *Bacteroides* infections in children, *J. Med. Microbiol.*, **43**, 92–98.