Taxonomy of the Bacteroides

I. Deoxyribonucleic Acid Homologies Among *Bacteroides fragilis* and Other Saccharolytic *Bacteroides* Species

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Strains of the "*Bacteroides fragilis* group" and other saccharolytic intestinal bacteroides were delineated by deoxyribonucleic acid homology. Of the 340 strains studied, about 90% belonged to any 1 of 10 major homology groups: the named species *B. fragilis*, *B. vulgatus*, *B. distasonis*, *B. thetaiotaomicron*, *B. ovatus*, *B. uniformis*, and *B. eggerthii*, a described group temporarily designated "subsp. a," and groups "3452-A" and "T4-1." Organisms belonging to the two latter groups had phenotypic similarities with and had not been previously differentiated from *B. distasonis*. Thirty more strains had no homology with the above-mentioned groups and appeared to be very heterogeneous when compared among themselves. There were two subgroups within the *B. fragilis* homology group. These subgroups had about 65 to 70% intergroup homology and about 80 to 90% intragroup homology. The *B. ovatus* and *B. thetaiotaomicron* homology groups had similar levels of heterogeneity, but they were more complex in that they appeared to have greater numbers of subgroups. The rest of the homology groups were quite homogeneous, usually having 80 to 90% intragroup homology.

Many of the organisms placed in the genus *Bacteroides* are commonly isolated from the digestive tracts of humans and other animals. These have been referred to as the "saccharolytic intestinal bacteroides" (21) or the "*B. fragilis* group" (1, 8), and they comprise a major portion of the bacterial population of the large intestine (11, 18). Some, notably *B. fragilis*, cause infections in humans and other animals (20).

Many of the strains on which the first descriptions of the named *Bacteroides* species were based are no longer extant and, in many cases, the original descriptions were rather incomplete. Therefore, the relationships of these organisms with currently recognized taxa can only be conjectured. Holdeman and Moore (10) discussed the similarities and differences of many of these "lost species" in the 8th edition of *Bergey's Manual*. Although the *B. fragilis* subspecies (10) were originally described as species, they were phenotypically very similar. All consisted of gram-negative rods, somewhat irregular in length, fermented many of the same carbohydrates, and produced similar fermentation products. Phenotypic traits usually used for bacterial identification include indole production and the fermentation of individual carbohydrates. It was because of their phenotypic homogeneity that Moore and Holdeman (17) placed these organisms in a single species, *B. fragilis*, consisting of five subspecies: *B. fragilis* subsp. *fragilis*, *B.*

fragilis subsp. *vulgatus*, *B. fragilis* subsp. *distasonis*, *B. fragilis* subsp. *thetaitaomicron*, and *B. fragilis* subsp. *ovatus*.

Preliminary deoxyribonucleic acid (DNA) homology data showed that the DNA diversity among representatives of these subspecies is of a degree usually considered to warrant differentiation at the species level (12), and we therefore recommended the reinstatement of these subspecies as species; neotype strains were proposed for those taxa for which type strains were not extant (6).

The purpose of this investigation was to delineate further the genetic groups of these saccharolytic bacteroides by means of their DNA homologies. We also planned to look for nucleotide similarities among a sufficiently large number of strains to determine whether correlations can be made between specific homology groups and phenotypic traits, such as nutritional requirements, fermentation of particular compounds, serological reactions, and phage-susceptibility patterns.

MATERIALS AND METHODS

Bacterial strains. Three hundred two strains of fermentative, non-pigmenting, anaerobic, gram-negative rods that produce succinic acid from fermentation of glucose were included in this study. Both clinical and fecal isolates were studied. To eliminate the possibility of including multiple isolates of the same strain, each strain within a homology group was isolated from a different person or, in a few cases, animal.

The strains have been designated herein by one of several numbering systems. Organisms whose strain numbers consist of arabic numerals are listed in Table 1. The majority of these strains were received from clinical laboratories for identification; others were received from other investigators or were isolated at the Anaerobe Laboratory, as indicated. Many additional strains were isolated by W. E. C. Moore and L. V. Holdeman during investigations of the flora of human feces. These were isolated from 10^8 , 10^9 , or 10^{10} dilutions of fecal material (11, 18). Strains whose designations begin with "A" or "J" were isolated from the feces of people from Africa or Japan, respectively, whereas those whose designations begin with "C" were from the feces of Japanese-Hawaiians. For these strain designations, the first number after the letter is

the person code, and the numbers after the hyphen refer to the isolate number. For example, C10-7 refers to the 10th volunteer and the 7th colony picked. All strain designations beginning with C10 are from the same fecal sample. Strain designations beginning with "B," "R," or "T" were from the feces of the three astronauts in the NASA Skylab Medical Experiments Altitude Test. Eight or nine fecal specimens from each astronaut, collected over 21 to 24 weeks (11), were cultured for predominant fecal bacteria. In these cases the letters refer to individuals, the first numbers after the letters refer to the sample numbers, and again the numbers after the hyphens are the isolate pick numbers. As an example, strain designation T4-1 means the first colony picked from a roll tube from the fourth fecal sample from individual "T." Other strain designations

Table 1. *Organisms used in the study that have arabic-numeral VPI designations*

VPI no.	Source and other designations ^a	Isolated from ^b	VPI no.	Source and other designations ^a	Isolated from ^b
<i>B. fragilis</i> type I					
2553 ^c	ATCC 25285		6195	VA Hospital Richmond, VA 227-10-3654	
	Sonnenwirth EN2				
	NCTC 9343	appendix abscess	6267-B	Corning, N.Y. 146	blood
0434	Prévot 670	acute appendicitis	6754	Baroness Erlanger Hosp., Chattanooga, TN 7475	peritoneal abscess
0439	Prévot 2342A	liver abscess			
0479	Prévot 1048A	liver abscess			blood
1521A	CDC 1294	wound	6805	Tucson Medical Ctr. Tucson, AZ	
1522	CDC 1267	blood			
1582	CDC 1313E	wound	6815	Dalton (MCV)	appendix
2362	Reinhold 274	liver abscess	6851	Mercy Hospital, San Diego, CA	lung tissue
2550	Fingold B11	ischio-rectal abscess			?
2554	Sonnenwirth EN3		6859-B	St. Raphael Hospital New Haven, CT 388	blood
	NCTC 9344	septic wound			
9556	Sonnenwirth FB-2	appendix abscess	6957	Forsyth Memorial Hosp., Winston-Salem, N.C.	blood
2557	Sonnenwirth JH-4	septic arthritis joint			
2633	Balows (LC) F-419-7	abdominal wound	8602	VPI	blood
			8604	VPI	feces
2758-A	Balows (LC) H-139-7	rectal abscess	8606	French Hospital, N.Y.	turkey gut contents
			8649	CDC 1261	?
3054	Baltimore City Hospital	blood	11666	Beerens E323	blood
		abscess			?
3156	Dalton (MCV)	blood culture			
3277	Dalton	pleurisy			
3389	Prévot 3053	?	2393	Beerens 12	<i>B. fragilis</i> type II
3390	Prévot 640-A	uterus	2360	Reinhold 234	?
3625	Dalton (MCV)	sub-phrenic abscess	2552	Sonnenwirth EN1	lung
4048	Prévot 2895	ovarian abscess			
4082	Prévot 2359	ascitic fluid	2647-J2	NCTC 8560	appendectomy
4128	Prévot 3020	appendectomy	3392	VPI	hog cecum
4147	Prévot 3953	?	4076	Prévot 4392	?
4361	Beerens 376	septicemia	4112	Prévot 2228	blood
4366	Prévot 2006		4117	Prévot 3748	buttock abscess
4489	Balows (LC) 1-198-8	rectal abscess	4225	Prévot 4026	?
				Prévot 3804	septicemia
<i>B. thetaiotaomicron</i>					
4509-B	Balows (SJH) J-22-8	rectum	5482 ^c	ATCC 29148	
4517	Balows (SJH) I-356-8	wound		NCTC 10582	
4736	Lambe IR426	surgical wound	0489	Werner E50	feces
4912	Lambe 164-058U	blood		ATCC 12290	infection after
4932	VA Hospital Richmond 244-44-1305	hip drainage	0633	CDC 1041A	appendectomy
		wound	0940	VPI 5MF95	peritoneal cavity
5001	Lambe 3R665		2579-A	Balows (LC) E314-7	feces
5383	ATCC 23745	pleural fluid	2808-B	Balows (LC) I-33-7	rectal abscess
	NCTC 10581				rectal abscess
5631	Gunderson Clinic LeCrosse, WI	mitral valve	3164-A	Balows (LC) K-231-7	rectal abscess
5650	New Hanover Hosp., Wilmington, N.C. 70-39	myocarditis	3388	Prévot 2080	perineal abscess
		?	3512	VPI	feces
5785	CDC 3820		5008	Dalton, MCV	hip drainage
5807	New Hanover Hosp., Wilmington, N.C. 244	blood	5377	Dalton, MCV 472	blood
			5961	Baltimore City Hosp., Baltimore, MD	leg ulcer
6001-A	Florida State Dept. of Health Jacksonville 894	abdominal drain	6212	Corning, N.Y. 3679	abdominal wound
			6396-C	Corning, N.Y. 446	abdominal incision
6057-B	Tucson Medical Ctr., Tucson, AZ	buttock drainage	7330	Lambe N-38-72D	stab wound
			8609	VPI 2357	
6059	St. Anthony Hospital, Terre Haute, IN 804	?		Reinhold No. 12	testicle abscess
			2302	VPI 13M	cecum
6123	Corning, N.Y. 3321	abdominal drainage	0911A	VPI 2MF95A	feces
			3051	Balows (UK) J94-4	

Rad) was added, and the suspension was shaken for 1 h on either a reciprocal or a rotary shaker at a speed just sufficient to keep the HA in suspension. The mixture was centrifuged for 2 to 3 min at $5,000 \times g$ in an SS-1 centrifuge (Sorvall). The lysate was returned to the flask. Eight milliliters of 0.05 M phosphate buffer was added to the centrifuge tube, and the HA was resuspended with the aid of a Vortex mixer. An additional 24 ml of 0.05 M phosphate buffer was dispensed with a Brewer automatic pipetting machine (BBL) into the HA suspension. After the HA was allowed to settle for a few minutes, the tubes were again centrifuged. This washing process was repeated until there was less than 0.05 absorbance at 270 nm, the absorption maximum of phenol. In practice this was usually six or seven times. The HA was then resuspended in 5 ml of 1 M phosphate buffer to desorb the DNA. After centrifugation, the supernatant containing the DNA was saved. The HA was washed once with distilled water and was returned to the lysate for a second cycle of DNA adsorption and purification. The two DNA samples from each lysate were pooled, passed through a glass filter (2.4 cm, 934AH, Reeve Angle) to remove remaining HA particles, and then dialyzed against 0.02 M NaCl. The DNA preparations were concentrated by ethanol precipitation and redissolved in 0.02 M NaCl- 10^{-3} M N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid (pH 7.0). The DNA (1.5 to 2.0 mg/ml) was then extracted by sonic oscillation two times, 30 s each time, using a sonic oscillator with a 0.375-inch (ca. 0.95-cm)-diameter tip and with the energy set at 60%. The DNA preparations were denatured by heating in a boiling-water bath for 5 min and then cooled rapidly by placing the tubes in ice. The DNA preparations were finally dialyzed against cold $2.2 \times$ standard saline citrate (SSC = 0.15 M NaCl, 0.015 M sodium citrate [pH 7.0]) adjusted to 1.5 mg/ml and stored in a freezer until used in the competition experiments.

G+C content of DNA. The guanine-plus-cytosine (G+C) contents of the DNA preparations were calculated by using the thermal melting points (T_m) of the DNA preparations (13, 15).

DNA immobilization and DNA homology experiments. The methods used for immobilizing DNA on nitrocellulose filters and for doing the DNA competition experiments were previously described (13).

Survey experiments were done on each DNA preparation to determine the homology group to which it belonged and to insure that the culture from which the DNA was isolated was not a mixture of these morphologically similar organisms. Such mixtures had high levels of homology to two reference DNA preparations that had little homology with each other. These survey experiments were dual label, and pairs of reference organisms having little or no homology with each other were used together. The DNA of one organism was labeled with ^3H , and the DNA of the other was labeled with ^{14}C . A mixture of both kinds of DNA (one-half the normal amount of each) was immobilized on the membrane. Duplicate reaction vials for each competitor DNA (75- μg amounts) were used. This enabled the screening of large numbers of DNA preparations against 10 reference DNAs in half the time and, more importantly, with less expenditure of DNA.

The incubation temperature used in the survey experiments was 62°C. For the complete homology experiments, the incubation temperatures were 25°C below the T_m of the reference DNA and ranged from 61.0 to 63.0°C. These incubation (reassociation) temperatures are listed in Table 2.

RESULTS

The interhomology values for the 16 reference strains used are given in Table 3. Data within the dashed lines of the table indicate those DNA preparations that have moderate to high (about 35% or greater) levels of nucleotide similarity.

DNA preparations from other strains that appeared to belong to this group on the basis of phenotypic properties were screened with the dual-label survey method to determine relatedness to the DNA of a reference strain from each of the major homology groups. DNA from about 90% of the strains showed high (about 60% or greater) homology with the DNA of one of the reference strains. Those strains showing high DNA homology with one of the reference strains were used as sources of competitor DNA with reference DNA of strains that were similar, either by homology studies (Table 3) or by phenotypic tests. Results of these studies are presented in Tables 4 through 13. The G+C contents of the DNAs from the competitor strains are also indicated in these tables. The G+C values ranged from about 40 to 46 mol%. These results are comparable to those (41 to 45 mol%) obtained by Sebald (19), who determined the G+C values of similar organisms by the use of hydrolysis and chromatographic separation.

The 81 strains of *B. fragilis* have DNA with an average G+C content of 42 mol%, and they can be separated into two closely related but

Table 2. Reassociation temperatures that were used for the various reference DNA preparations

Taxon	VPI no.	Reassociation temperature(°C)
<i>B. fragilis</i>	2553	61.5
	2393	61.5
<i>B. thetaiotaomicron</i>	5482	61.5
	2302	61.5
<i>B. ovatus</i>	0038	61.0
	3524	61.0
	C11-2	61.0
'3452-A'	3452-A	61.0
<i>B. uniformis</i>	0061	63.0
	C20-25	63.0
'subsp. a'	B5-21	63.0
<i>B. eggerthii</i>	B8-51	63.0
<i>B. vulgatus</i>	4245	61.0
	B2-4	61.0
<i>B. distasonis</i>	4243	62.0
'T4-1'	T4-1	62.0

Table 3. Interstrain homology values among the DNA reference organisms

Strain no.	<i>B. fragilis</i> 2553	2393	<i>B. thetaiotaomicron</i> 5482	2302	<i>B. ovatus</i> 0038	3524	C11-2	'3452-A' 3452-A	<i>B. uniformis</i> 0061	C20-25	'subsp. a' B5-21	<i>B. eggerthii</i> B8-51	<i>B. vulgatus</i> 4245	B2-4	<i>B. distasonis</i> 4243	'T4-1' T4-1
2553	100	69	21	36	15	13	22	10	21	20	26	18	10	5	5	15
2393	68	100	19	29	4	12	19	14	18	26	29	18	9	11	17	13
5482	28	28	100	61	40	43	39	36	22	26	21	23	12	10	7	18
2302	21	30	62	100	42	43	36	47	23	28	27	21	11	28	5	16
0038	24	15	39	39	100	80	64	31	17	20		20	8	2	4	8
3524	9	17	40	44	75	100	66	35	15	21	21	19	8	0	0	15
C11-2	20	22	34	40	65	68	100	43	13							
3452-A	23	20	26	35	37	34	36	100	11	21	20	18	0	5	3	4
0061	17	14	22	14	9	11	13	6	100	81	38	36	14	10	6	15
C20-25	9	24	27	16	23	12	14	20	82	100	38	36	18	9	14	16
B5-21	28	23	19	27	25	16	23		30	28	100	54	19	29	12	19
B8-51	28	25	23	17	12	18	13	30	34	36	49	100	20	26	8	15
4245	20	14	9	13	8	0	16	4	19	14	19	14	100	79	9	20
B2-4	6	16	9	17	2	1	8	2	0	17	20	6	77	100	0	6
4243	18	8	5	4	3	0	0	3	10	8	5	3	7	0	100	20
T4-1	13	12	6	16	7	1	1		11	11	18	6	3	19	23	100

distinct homology groups (Table 4). The majority (67) of the strains were most closely related to reference strain 2553 (= ATCC 25285), the neotype strain of *B. fragilis*. This group was designated *B. fragilis* homology group I. *B. fragilis* homology group II consisted of 14 strains, the reference strain being 2393. The reciprocal homology values of the DNA preparations from the two groups with the reference DNA preparations were very close, such that all of the DNA preparations clearly belonged to one or the other homology group.

The *B. thetaiotaomicron*, *B. ovatus*, and "3452-A" homology groups were interrelated at about the 30 to 40% homology level (Tables 5 through 7). The average G+C content of the DNAs from the strains of the *B. thetaiotaomicron* homology group, like that of *B. fragilis*, was 42 mol%, whereas the G+C content of DNA preparations from strains of the *B. ovatus* and "3452-A" homology groups averaged about 41 mol%.

The DNA heterogeneity among the strains of *B. thetaiotaomicron* (Table 5) was also similar to that observed with *B. fragilis* in that the homology between the two reference strains of *B. thetaiotaomicron* (5482 and 2302) was 61 to 62%. Most of the DNA preparations (30 to 34) had high homology with 5482, the neotype strain. The DNA from only one strain (T1-37) had what one would consider high homology (82%) with the DNA from 2302. DNAs from the last two strains listed in Table 5 (group III) have a lower level of homology with both reference DNA preparations.

Homology results of the *B. thetaiotaomicron* strains with the *B. uniformis* reference strain

(0061) were included in Table 5 because most organisms in the *B. uniformis* homology group (see Table 8) had been phenotypically identified as strains of *B. thetaiotaomicron*. However, DNAs from the *B. thetaiotaomicron* strains have only low homology with the DNA of *B. uniformis* strain 0061.

The first two of the three *B. ovatus* reference strains (0038 and 3524) had nucleotide sequence similarities of 75 to 80% with each other (Table 6). The DNA preparation from 21 of the strains (0435 through T4-7, Table 6) had high homology with both 0038 (the type strain of *B. ovatus*) and 3524, although the DNAs from more of these strains had higher homology values with 3524 DNA. The last 18 strains listed in Table 6 (group II) had homologies in the range of 62 to 75% to both reference strains. This represents a somewhat higher proportion of strains with this level of homology than had been found in the *B. fragilis* and *B. thetaiotaomicron* homology groups. Therefore, a third reference organism (C11-2) was selected to test for homogeneity among these latter strains. One strain (C33-13) was 82% homologous to C11-2, and six strains were 76 to 77% homologous (Table 6). The rest of the strains had similar levels of homology to all three reference DNA preparations.

The organisms found in the "3452-A" homology group (Table 7), unlike those in the *B. thetaiotaomicron* and *B. ovatus* groups, were quite homogeneous in that DNA preparations from all of the strains had from 84 to 96% homology with reference strain 3452-A. Although many of these strains had been phenotypically identified as belonging to *B. distasonis*, they had little or no homology with *B. distasonis*

Table 4. DNA homologies of strains of *B. fragilis* with reference DNA of *B. fragilis* group I (2553) and *B. fragilis* group II (2393)

Competitor DNA strain no.	mol% G+C	% Homology with		Competitor DNA strain no.	mol% G+C	% Homology with	
		2553	2393			2553	2393
Group I							
2553	42	100	69	6195	43	88	72
0434	42	87	72	6267-B		90	71
0439	42	88	69	6754	43	92	74
0479	43	88	67	6805	42	89	74
1521-A	43	84	75	6815	44	90	76
1552	41	91	66	6851	43	90	71
1582	42	89	69	6859-B	43	89	70
2362		86	73	6957	42	91	71
2550	42	86	70	8602	43	85	75
2554	44	85	68	8604	42	86	76
2556	42	89	69	8606	43	88	72
2557	43	85	71	8649	44	86	78
2633	43	87	69	11666		92	77
2758-A	42	87	68	A1-22		91	75
3054	42	92	75	A7-5A	44	94	61
3156	43	88	75	A9-7	43	93	64
3277	42	89	74	B2-22	42	87	70
3389		88	70	C10-7		87	77
3390	44	90	76	C20-6	42	80	72
3625	42	88	70	C45-31B		92	65
4048	43	86	72	C46-29	44	90	64
4082	43	91	74	C50-3	42	94	75
4128	43	87	73	J6-3		96	65
4147	42	90	76	J14-5		94	68
4361	43	91	73	T4-43	42	92	71
4366	42	94	68				
4489	43	85	74				
4509-B	42	87	73	Group II			
4517	43	86	72	2393	42	68	100
4736	42	89	72	2360	43	68	90
4912	43	94	71	2552	43	68	89
4932	41	89	66	2647-J2	42	68	85
5001	43	79	66	3392	42	72	93
5383	43	91	67	4076	43	72	90
5631	42	89	69	4112	43	68	92
5650	42	89	75	4117	41	64	80
5785	41	87	73	4225	43	65	94
5807	42	92	74	A3-18B		70	97
6001-A	44	88	77	A10-2B	42	68	87
6057-B	43	87	72	A11-24B	42	67	87
6059	43	88	71	C40-1	41	69	84
6123	42	89	74	C48-32	41	72	88

reference DNA (strain 4243).

The results from another cluster of three interrelated homology groups—*B. uniformis*, "*B. fragilis* subsp. a" (18), and *B. eggerthii* (9)—are presented in Tables 8, 9, and 10. The DNA from these organisms contains 45 to 46 mol% G+C. Strains of *B. uniformis* (Table 8) comprised the largest group (27 strains) and had 30 to 40% DNA homology with the "subsp. a" (B5-21) and *B. eggerthii* (B8-51) reference strains. Organisms in the "subsp. a" and *B. eggerthii* homology groups had about 50% intergroup homology (Tables 9 and 10), and both had 30 to 40% homology to the *B. uniformis* reference strain (0061). The strains within each of these homology groups, like the "3452-A" homology group, were rather homogeneous, in that the majority of the strains had 80% or more DNA homology

with the reference DNA preparations representing that group.

All of the organisms in the *B. uniformis* group (Table 8) were phenotypically very similar to the strains of *B. thetaiotaomicron* when examined by the usual tests (8). However, there were only low levels of DNA homology between the respective reference strains (Table 3), and competitor DNA preparations of the *B. thetaiotaomicron* strains had little homology with DNA of 0061, the reference organism of *B. uniformis* (Table 5). Moreover, the G+C content of the DNA of *B. thetaiotaomicron* was 42 mol%, and that of *B. uniformis* was 46 mol%.

DNA preparations from the *B. vulgatus* homology-group strains had an average of about 41 mol% G+C. The 48 strains in this homology group (Table 11) were also rather homogeneous

Table 5. DNA homologies of strains of *B. thetaiotaomicron* with reference DNA of *B. thetaiotaomicron* (5482 and 2302), *B. ovatus* (0038 and 3524), '3452-A' group (3452-A), and *B. uniformis* (0061)

Competitor DNA strain no.	mol% G+C	Percent homology with					
		<i>B. thetaiotaomicron</i> 5482	<i>B. thetaiotaomicron</i> 2302	<i>B. ovatus</i> 0038	3524	'3452-A'	<i>B. uniformis</i> 0061
Group I							
5482	42	100	61	40	43	36	28
0489	42	82	69	41	38	46	25
0633	42	80	59				31
0911-A	42	74	68	32	32	36	9
0940-1	43	91	62	44	48		
2579-A	42	83	65	37	46	53	27
2808-B	43	93	68	45	46	49	29
3089	42	75	53	28	32	38	12
3164-A	42	81	61	33			19
3388	42	83	72				
3443	41	76	59	25	36	44	14
3512	42	87					
5008	43	81	62	40	46	50	33
5377	42	83	60	40	47	49	28
5951	42	80	62	37			25
6212	42	91	66	46	49		32
6396-C	43	90	59	37	44		26
7330	41	86	57	40	40	46	
8609	42	82	57	36	34		6
B1-46	41	85	64	38	46		24
C9-11	43	86	55	42	39	46	3
C11-15	41	92	58	40	37	47	19
C14-5	41	85	56	36	39		13
C20-22		77		22		33	5
C22-15		86	56	39	35	45	13
C41-27	40	81	67	38		41	
C54-11		92	66	33	38	44	
J4-7		83	69	35		41	
J11-23		86	66	39		41	
J19-34-B	42	87		35	42	47	
Group II							
2302	42	62	100	42	43	47	22
T1-37	42	59	82	30	33	35	18
Group III							
3051	43	66	41	15	25	26	4
C51-28	42	55		37		43	

Table 6. DNA homologies of strains of *B. ovatus* with reference DNA of *B. ovatus* (0038, 3524, and C11-2), *B. thetaiotaomicron* (5482), and '3452-A' group (3452-A)

Competitor DNA strain no.	mol% G+C	Percent homology with				
		<i>B. ovatus</i> 0038	3524	C11-2	<i>B. thetaiotaomicron</i> 5482	'3452-A'
Group I						
0038	40	100	80	64	39	31
3524	40	75	100	66	40	35
0435	41	75	86	66	50	52
2822	40	72	75	58		
2828-A	42	77	95	67	40	52
3049	40	75	85			
3794-B	41	77	93	57	34	35
4101	40	78	84	66	47	47
8605	40	81	97	41	47	
A10-48A3	41	90	91	58	36	48
A13-5A	40	93	88	72	37	46
C1-45	41	83	93	70	46	51
C8-14	41	71	80	62	38	40
C16-22	40	74	96	58	37	43
C41-35	40	76	88	65	37	44
C43-43	43	77	98	64	31	44
C48-5	39	82	89	65	38	46
C54-15	42	79	100	67	43	46
J7-44A		75	84	61	33	42
J8-15		79	99	67	38	42
J18-9	39	74	87	31	46	
R3-39	41	77	99	65	35	47
T4-7	39	78	85	71	44	46
Group II						
1900-C-1	41	71	70	70	40	51
C2-26	41	70	70	77	36	45
C11-2	40	65	68	100	34	43
C12-31	40	66	77	40		44
C24-33		64	68	76	40	
C33-13		62	63	82	41	
C40-50-A	40	64	69	70	36	43
C43-44-B	40	66	72	72	38	42
C45-43	42	65	68	69	32	41
C50-15B	40	73	66	41	48	
C51-23	41	66	69	77	33	45
C52-9A	40	68	70	73	36	46
B4-11	39	68	75	60	32	44
J2-6		68	71	76	35	44
J15-5	40	65	72	67	30	46
J20-44	40	68	72	73	35	45
J22-44	41	65	66	77	36	43
T2-21	42	65	62	72	34	34

Table 7. DNA homologies of '3452-A' strains with reference DNA of '3452-A' group (3452-A), *B. thetaiotaomicron* (5482), *B. ovatus* (0038), and *B. distasonis* (4243)

Competitor DNA strain no.	mol% G+C	Percent homology with			
		'3452-A'	<i>B. thetaiotaomicron</i> 5482	<i>B. ovatus</i> 0038	<i>B. distasonis</i> 4243
3452-A	40	100	26	37	3
2308	41	90	34	37	12
4227	41	84			5
8608	40	89	32	30	0
B6-11	42	93	37	42	19
C7-8	41	90	37	35	4
C8-12	41	90	35	33	5
C10-2	40	88	39	45	7
C14-3	41	92	39	37	4
C15-2	41	90	38	34	12
C27-54	42	90	45	41	21
C48-1	41	90	34	36	11
C51-18	41	93	34	35	
J7-54		94	31		
J14-14		91	33		
J15-29A	40	96	33	34	
OC-9	40	91	52	41	10
R2-37B	40	91	39	37	3
S2-4	42	91	32	38	12
S4A-53	42	90	34	36	11
T1-12	41	89	28	34	4

Table 8. DNA homologies of *B. uniformis* strains with reference DNA of *B. uniformis* (0061 and C20-25), *B. fragilis* subsp. a' (B5-21), and *B. eggerthii* (B8-51)

Competitor DNA strain no.	mol% G+C	Percent homology with			
		<i>B. uniformis</i> 0061	C20-25	'subsp. a' B5-21	<i>B. eggerthii</i> B8-51
0061	46	100	82	38	36
C20-25	45	81	100	38	36
0909	47	82	84	38	36
3537		77	79	37	35
3699	45	78	76	38	35
6387	46	83	87	38	36
S444-A	45	72	79	33	39
8601	46	68	72	31	32
11227		82		39	29
A7-10A	45	72	77	34	36
A13-47	45	84	84	40	34
B1-15	48	80	78	29	37
C1-10	46	80	83	34	40
C7-17	46	88	79	31	34
C8-30	47	95	89		42
C17-3	47	74	87	33	38
C46-14		82	85	41	
C50-6	45	86	94	39	34
C51-27	46	85	88	37	29
C54-1	45	92	97	39	36
J4-2		77	77	42	41
J8-10		70	72	29	37
J15-53	46	78	78	40	33
OC-22A	46	85	78	37	34
R5-33	46	78	79	33	35
T1-1	47	84	81	33	43

in that DNA from all but one (C48-14) had about 80% homology with DNA from both reference strains. The DNA from C48-14 had only about 60% homology with the reference DNA preparations.

The 15 *B. distasonis* DNA preparations averaged 44 mol% G+C, and the homology with 4243, the type strain, ranged from 77 to 91% (Table 12). This is an interesting group of organisms in that many that were phenotypically identified as belonging to *B. distasonis* had little or no homology to the reference strain. For example, many of the "3452-A" organisms had been identified as strains of *B. distasonis*, but the organisms in the *B. distasonis* homology group have little or no homology to reference strain 3452-A (Table 12).

The 10 organisms in the "T4-1" homology group (Table 13) also had been identified as

Table 9. DNA homologies of 'subsp. a' strains with reference DNA of *B. fragilis* subsp. a' (B5-21), *B. eggerthii* (B8-51), and *B. uniformis* (0061)

Competitor DNA strain no.	mol% G+C	Percent homology with		
		'subsp. a' B5-21	<i>B. eggerthii</i> B8-51	<i>B. uniformis</i> 0061
B5-21	46	100	54	32
A13-46	45	76	49	31
C1-23	46	81	58	29
C2-39		86	54	27
C8-19	46	79	62	31
C12-29	47	74	53	29
C16-26B	45	79	56	34
C19-3	45	82	55	38
C46-33	44	76	52	39
C49-2	46	84	59	28
C51-6	46	84	57	27
C52-51A	46	94	49	26
C54-2	45	84	55	33
J7-23A		82	56	35
J8-20		74	50	37
J16-24	45	84	53	29
J19-43	43	81	52	27
J20-9	44	84	54	28

Table 10. DNA homologies of *B. eggerthii* strains with reference DNA of *B. eggerthii* (B8-51), *B. fragilis* subsp. a' (B5-21), and *B. uniformis* (0061)

Competitor DNA strain no.	mol% G+C	Percent homology with		
		<i>B. eggerthii</i> B8-51	'subsp. a' B5-21	<i>B. uniformis</i> 0061
B8-51	45	100	49	31
C18-8	45	87	53	30
C19-14	44	86	58	33
C24-44		88	50	34
C31-22	43	99	51	32
S1A-52		82	49	30
T5-42-B	46	80	57	35
X3-31-1A	44	83	57	25

Table 11. DNA homologies of *B. vulgatus* strains with reference DNA of *B. vulgatus* (4245, B2-4)

Competitor DNA strain no.	mol% G+C	Percent homology with		Competitor DNA strain no.	mol% G+C	Percent homology with	
		4245	B2-4			4245	B2-4
4245	41	100	79	C28-29	40	85	85
B2-4	40	77	100	C43-46B	40	86	83
0959	41	84		C45-21	41	80	82
2277	41	87	82	C46-17	41	81	78
2365	41	86	80	C47-46A	41	83	83
2736-B	41	81	79	C49-4	41	79	82
3520	40	79	76	C50-4	41	74	73
4025	41	80	81	C52-27A	42	81	85
4506	41	86	84	C54-10	42	76	76
5710	41	87	76	C55-40	41	82	83
6186	41	82	82	J1-42A		80	81
6266A	41	81	81	J2-2		81	78
6598-B	41	80	78	J3-5B	41	80	80
A12-49A	40	76	76	J8-62A	40	84	80
A16-19	40	86	80	J15-9	40	81	80
A19-46A		84	81	J17-22	40	81	82
C1-13	42	78	83	J18-14B	40	81	82
C6-7		77	76	J20-4	41	81	81
C7-2	41	86	75	J21-28	41	83	80
C9-38	41	88	78	J22-25		86	81
C10-6	40	84	81	OC-13	41	85	84
C11-25	40	87	85	R1-5	40	83	78
C14-18	40	80	79	T1-8	40	79	77
C16-16	40	85	85	C48-14	41	61	58

members of *B. distasonis*. The G+C of the DNA from these organisms averaged 44 to 45 mol%, values very similar to those of the *B. distasonis* strains (Table 12). However, in the survey experiments, DNA from the strains in the "T4-1" group had little or no homology with the *B. distasonis* reference strain. The "T4-1" homology group was quite homogeneous in that DNA from five of nine heterologous strains had greater than 90% homology with the reference DNA.

DISCUSSION

The purpose of this investigation was twofold: first, to delineate into homology groups the genetically distinct groups within some of the saccharolytic bacteroides, and second, to investigate a large enough number of strains so that the range of diversity of nucleotide sequences and of phenotypic characteristics within each homology group could be determined. This paper is concerned with the DNA homology results; the more detailed phenotypic analysis of strains within these homology groups is dealt with in an accompanying paper (14).

The method of strain selection could have a great bearing on the heterogeneity (or homogeneity) of the results obtained in both the DNA homology and the phenotypic studies, and it will therefore be discussed in some detail. To avoid

Table 12. DNA homologies of *B. distasonis* strains with reference DNA of *B. distasonis* (4243) and '3452-A' group (3452-A)

Competitor DNA strain no.	mol% G+C	Percent homology with	
		<i>B. distasonis</i> 4243	'3452-A' 3452-A
4243	44	100	3
0052	44	78	0
A12-1	44	82	
B1-20	43	81	6
C1402	45	89	10
C18-7	45	80	7
C19-17	44	91	11
C21-1			
C30-45	44	91	8
C33-3	45	88	13
C50-2	44	89	
J15-49A	43	77	
S1-35	44	88	15
S6A-50	43	88	8
T3-25	43	88	3

Table 13. DNA homology of 'T4-1' strains with reference DNA of T4-1

Competitor DNA strain no.	mol% G+C	Percent homology with T4-1
T4-1	44	100
2628P	46	91
A19-28B	45	96
B8-5	44	83
C16-2	45	97
C43-5	43	91
C54-23	45	98
J8-26		
J20-53	45	70
R3-5	44	87

any bias in strain selection because of prior knowledge of phenotypic results, we would have preferred to establish the homology groups and then determine the phenotypic properties of the groups. However, from a practical standpoint, it was not possible to take this approach. Our first interest was to study *B. fragilis* and related species. Therefore, the reference strains initially selected were the type or neotype strains of *B. fragilis* (VPI 2553 = ATCC 25285), *B. thetaiotaomicron* (VPI 5482 = ATCC 29148), *B. ovatus* (VPI 0038 = ATCC 8483), *B. vulgatus* (VPI 4245 = ATCC 8482), and *B. distasonis* (VPI 4243 = ATCC 8482). ATCC 8492 (=VPI 0061, labeled *B. thetaiotaomicron* and later identified as *B. uniformis*) also was included in the original set of reference strains.

For comparison with these reference organisms, 150 strains were selected from our collection. These included those that were phenotypically similar to the reference strains as well as many that were somewhat variant and referred to as "no-good-fits." Many of these strains had been isolated in clinical or other research laboratories and sent to the Anaerobe Laboratory either for identification or for comparison studies (Table 1). Organisms identified as members of *B. fragilis* were the most numerous in the collection, and 50 were randomly selected for inclusion in this study. For some of the other groups, most of the strains in the collection were used.

Later in the study, organisms that were being isolated by W. E. C. Moore and L. V. Holdeman from human feces were also included. The method of selection of these strains was more complex and was altered during the course of the investigation. Initially we studied more than one isolate from a fecal specimen if there appeared to be phenotypic variation among the strains of a species. Also, strains from different fecal specimens of the same individual were used. However, we observed that when organisms belonging to a given homology group were isolated from a single fecal sample, or from several fecal samples from the same person, the DNA homology of many of these isolates appeared to be absolutely identical, indicating that the intestinal tract of an individual may be populated with bacterial strains unique to that individual. For example, DNA from most of the *B. vulgatus* strains listed in Table 11 ranged between 75 and 85% homology with the two reference DNA preparations. The one reference strain (B2-4) was isolated from the feces of an astronaut (11). When DNA preparations from other *B. vulgatus* strains isolated from other fecal specimens from the same astronaut were tested against the B2-4 DNA, most were 98 to

100% homologous with it. This phenomenon has been observed in several other homology groups, and a more detailed investigation is in progress. However, since the aim of the present study was to obtain a cross section of both phenotypic and DNA homology diversity, we have included in this report no more than one isolate, for each homology group, from one individual.

More than 300 strains were tested for homology with the initial six reference DNA preparations by using the dual-label survey experiments. The survey experiments were also very useful for detecting the occasional mixed culture among these morphologically similar organisms. Since the competition curve is exponential, even a small amount of contaminating DNA in a competitor DNA preparation could compete substantially to a second supposedly dissimilar DNA.

About 90 strains did not show any significant homology with DNA of the first six reference strains tested. Reference DNA was then prepared from some of these strains, and the strains were retested with the new reference DNA. Forty-nine strains belonged to one of four additional homology groups: *B. eggerthii* and three unnamed strains designated "subsp. a," "3452-A," and "T4-1." An additional 39 strains phenotypically similar to these 10 homology groups had little or no homology with reference strains used in the survey experiments. Most of these strains were phenotypically similar to *B. vulgatus*, *B. distasonis*, *B. thetaiotaomicron*, and *B. fragilis*. Several additional reference strains were selected from this group of organisms, but DNA from only a few strains had high levels of homology with DNA from these new reference strains, and the results from these studies are not included in this report. In all, 85 to 90% of the saccharolytic bacteroides selected for study were found to belong to one of these 10 homology groups.

The sizes of the homology groups ranged from 81 strains for *B. fragilis* to 8 strains for *B. eggerthii*. The size of a homology group, together with the source of the organisms within it, reflects the incidence of these organisms in human clinical specimens and feces (Table 14), since about an equal number of strains from each source was included in this study. The source of a large proportion of the *B. fragilis* strains was clinical specimens, whereas the majority of the *B. vulgatus* strains were fecal isolates. Similar distributions of species have been reported by other investigators (11, 17, 21). It was interesting that a rather high proportion of the *B. thetaiotaomicron* strains was isolated from infections whereas the majority of the phenotypically similar *B. uniformis* strains was iso-

lated from intestinal contents or feces (Table 14). If one considered only the fecal isolates, the number of strains per homology group reflected their relative incidence in 10^8 to 10^{10} dilutions of feces although not their relative numbers within those dilutions. Strains of *B. ovatus*, as an example, were found in 30 fecal specimens (Tables 1 and 6), but *B. eggerthii* strains were found in only 8 (Table 10).

The *B. fragilis* strains fit into two homology clusters (Table 4). Greater than 80% of them have high levels of homology with the neotype strain of the species (VPI 2553). This group of strains has been designated *B. fragilis* group I. The second reference strain (VPI 2393) was arbitrarily selected from several strains that had only about 65 to 70% homology with strain 2553. The DNA preparations from these strains were also quite homogeneous in that their homologies with 2393 ranged from 80 to 97%. These have been designated *B. fragilis* group II. The thermal stability profiles of homologous and heterologous duplexes have been determined for some of these organisms (12). The temperature at which 50% of the duplexes have disassociated is the $T_{m(e)}$ value. The difference between the $T_{m(e)}$ value of homologous versus heterologous duplexes is the $\Delta T_{m(e)}$ value, which is a measure of the proportion of bases within the duplexes that do not form hydrogen bonds. Heterologous duplexes formed between DNA preparations from strains of the two *B. fragilis* homology groups had $\Delta T_{m(e)}$ values that ranged from 7.2 to 9.5°C, whereas the values for heterologous duplexes formed between DNA preparations from strains within each homology group varied from 0.0 to 1.2°C (12). These results show that there has been a substantial divergence in the arrangement of the DNA nucleotides between the organisms of these two groups. Following a previous proposal (12) on taxonomic groupings, these would be considered genosubspecies.

Although the majority of the *B. fragilis* strains (Table 4) belong to homology group I, similar ratios of the two groups were found among both clinical and fecal isolates (Tables 1 and 14). This suggests that strains from both groups may have similar pathogenicities. Therefore, both groups need to be considered in the development of serological or bacteriophagic identification procedures.

B. thetaiotaomicron (Table 5), *B. ovatus* (Table 6), and the "3452-A" homology group (Table 7) all have partial homology. Most of the intergroup homology values were in the range of 30 to 40%. The phenotypic characteristics of these groups will not be dealt with in any detail here, but some general observations will be discussed.

Strains phenotypically identified as *B. thetaiotaomicron* include the *B. thetaiotaomicron* strains (Table 5), *B. uniformis* strains (Table 8), and *B. ovatus* strains (Table 6). The fermentation of mannitol and the oval shape of cells have been used to differentiate *B. ovatus* from *B. thetaiotaomicron*. The type strain of *B. ovatus* does utilize mannitol, but this was true only of it and an additional four of the 41 strains listed in Table 6. Most of the strains that were phenotypically identified as *B. ovatus* were *B. ovatus*, but nearly half of the *B. ovatus* strains in Table 5 were phenotypically identified as *B. thetaiotaomicron* (W. E. C. Moore, personal communication). Therefore, the cell morphology is helpful but somewhat inconsistent. The third member of these interrelated homology groups, the "3452-A" group (Table 7), was phenotypically distinct in that all of the strains did not produce indole whereas most strains of *B. thetaiotaomicron*, *B. uniformis*, and *B. ovatus* did.

The majority of the *B. thetaiotaomicron* strains (Table 5) had high levels of homology with the neotype strain of the species. Differences in nucleotide sequences between the reference strains—2302 and 5482—were similar to those between the two *B. fragilis* reference strains. The relationships between the *B. thetaiotaomicron* subgroups were not as clear-cut as for the two *B. fragilis* subgroups since only one additional strain had high homology with 2302, and the homology values for a couple of other strains were rather low for both reference strains. It therefore would be premature to recommend genosubspecies designation until more of these strains have been isolated and their relative importance has been ascertained.

Two of the *B. ovatus* DNA reference strains, the type strain (VPI 0038) and VPI 3524, were quite closely related (Table 6). It was of interest that in Table 6 the 21 strains listed after the two reference strains had generally higher levels of homology with strain VPI 3524, indicating that

Table 14. Distribution of strains in each homology group in infections and intestinal or fecal contents

Homology group	No. of strains	Percent of strains		
		Isolated from		Source not known
		Infections	Intestinal or fecal contents	
<i>B. fragilis</i> I	67	69	21	10
<i>B. fragilis</i> II	14	36	43	21
<i>B. thetaiotaomicron</i>	34	44	56	0
<i>B. ovatus</i>	41	15	83	2
'3452-A'	21	10	90	0
<i>B. uniformis</i>	26	4	88	8
'subsp. a'	18	0	100	0
<i>B. eggerthii</i>	8	0	100	0
<i>B. vulgatus</i>	48	15	85	0
<i>B. distasonis</i>	15	0	100	0
'T4-1'	10	0	100	0

it may be a better representative of the group. Five strains had from 97 to 100% homology with reference strain 3524, which was a level of homogeneity among independently isolated strains not found in the other homology groups. The homology values for the last 18 strains (group II) listed in Table 6 ranged from 62 to 75% with these two reference strains. Therefore, a third reference organism (C11-2) was selected from these latter strains with the thought that it might represent another subgroup. However, only one strain (C33-13) had what one would consider high homology (82%), and an additional six had 76 to 77% homology with strain C11-2. The latter values are in the inconclusive homology range, where they may be characteristic of either genovarieties or genosubspecies (12). Thermal stability profiles of heterologous duplexes should be measured to more clearly determine their relationships. Even if these strains do represent genovarieties of reference strain C11-2, they account for less than half of those strains that have lower levels of DNA homology with reference strains 0038 or 3524. There appeared to be more nucleotide sequence heterogeneity among the *B. ovatus* strains than among strains in the other homology groups.

The "3452-A" homology group (Table 7) had not been phenotypically recognized as a species although the homology data justify such recognition. A majority of the strains had been phenotypically identified as *B. distasonis* (W. E. C. Moore, personal communication). The DNA base composition of the strains averaged 41 mol% G+C, and all of the strains had high homology (84 to 96%) to the 3452-A reference strain.

There was a second set of interrelated homology groups. Organisms of *B. uniformis* (Table 8) have 30 to 40% homology to organisms in the "subsp. a" and *B. eggerthii* homology groups (Tables 9 and 10). Organisms in the latter two groups have about 50% intergroup homology. The DNA preparations from organisms in these groups contained 45 to 46 mol% G+C.

Isolates belonging to the *B. uniformis* homology group appeared to be as commonly isolated as were strains of *B. thetaiotaomicron* as judged by the similar number of organisms in each homology group. Most of the strains had high homology with the DNA of the *B. uniformis* reference organisms (0061 and C20-25), with only 8601, A7-10A, and J8-10 being in the 68 to 72% homology range.

The majority of the organisms in the "subsp. a" homology group (Table 9) had been phenotypically identified by W. E. C. Moore as "*B. fragilis* subsp. a," for which a brief description

has been published (9). The DNA preparations have an average of 45 mol% G+C, and all have high homology to the DNA reference strain B5-21.

The species *B. eggerthii* was recently described by Holdeman and Moore (9). All of the strains of this species that were tested, including the type strain (T5-42B), have 80 to 99% homology to the DNA of the reference strain (B8-51) (Table 10).

B. vulgatus is one of the major organisms found in the colon (9, 11), and as a result a large proportion of the strains used in this study was isolated from feces (Tables 1 and 11). The DNA preparations averaged 41 mol% G+C, and all but one of the preparations had high homology to DNA from both the type strain of the species (4245) and the second reference strain (B2-4). The last strain listed in Table 11—C48-14—had only 61 and 58% homology to the two reference organisms. Strain C48-14 may represent a subgroup similar to those found in some of the other homology groups, but since it represents only one out of 48 strains in the present study, it is probably rare.

The DNA from organisms fitting the phenotypic description of *B. distasonis* has turned out to be very heterogeneous. Twenty of the strains in the "3452-A" homology group were identified as *B. distasonis* as were the 10 organisms in the "T4-1" homology group (Table 13). Also, 15 of 39 organisms that did not fit in any of the homology groups were identified as *B. distasonis*. The 16 strains in the *B. distasonis* homology group had DNAs which averaged 44 mol% in G+C content and which ranged from 77 to 91% in homology with DNA from the type strain of the species (strain 4243). All of the strains in the *B. distasonis* homology group were isolated from feces, which suggests that this group may be of little clinical significance.

The "T4-1" homology group had phenotypic traits that are distinct from those of *B. distasonis*. Some of the characters described as variable for *B. distasonis* (8) separate the two homology groups. For example, most of the *B. distasonis* strains fermented cellobiose and were catalase positive, but the "T4-1" strains were negative with respect to these traits. Strain T4-1 was quite distinct from all of the other reference strains used in this study in that it did not incorporate exogenous [³H]adenine into its DNA. Exogenous [³H]thymidine was incorporated, but only to a very low specific activity.

This study has shown that the more commonly isolated clinical and saccharolytic intestinal bacteroides comprise 10 major DNA homology groups. The two groups that were most

frequently isolated from clinical specimens are *B. fragilis* and *B. thetaiotaomicron*. There were fewer clinical isolates among strains of *B. ovatus*, *B. vulgatus*, *B. uniformis*, and "3452-A" than among *B. fragilis* and *B. thetaiotaomicron*.

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REPRINT REQUESTS

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