APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1986, p. 385–390 Vol. 51, No. 2
0099-2240/86/020385-06$02.00/0
Copyright © 1986, American Society for Microbiology

# Enumeration of Polysaccharide-Degrading *Bacteroides* Species in Human Feces by Using Species-Specific DNA Probes

ALEX P. KURITZA, PEG SHAUGHNESSY, AND ABIGAIL A. SALYERS*
*Department of Microbiology, University of Illinois, Urbana, Illinois 61801*

Received 5 August 1985/Accepted 19 November 1985

**DNA probes that are specific for each of five predominant species of human colonic *Bacteroides* (*B. thetaiotaomicron*, *B. uniformis*, *B. distasonis*, "*Bacteroides* group 3452-A", and *B. ovatus*) were used to detect and enumerate these species in fecal samples from two adult volunteers. These five species are capable of fermenting many of the complex polysaccharides that are thought to be sources of carbon and energy for bacteria in the colon. Estimates for the concentrations of some of these species in feces have not been previously available because of the difficulties in differentiating colonic *Bacteroides* spp. by conventional biochemical tests. Our results indicate that all the species except *B. ovatus* were present in high numbers (>$10^9$/g [dry weight]) in the feces of both volunteers. However, the concentrations of the more versatile polysaccharide-degrading species within this group of organisms ($7.6 \times 10^9$ to $12.0 \times 10^9$/g [dry weight] for *B. thetaiotaomicron*; $2.9 \times 10^9$ to $6.3 \times 10^9$/g [dry weight] for "*Bacteroides* group 3452-A") did not differ significantly from the concentrations of less versatile polysaccharide-degrading species ($1.2 \times 10^{10}$ to $2.0 \times 10^{10}$/g [dry weight] for *B. uniformis*; $5.8 \times 10^9$ to $8.4 \times 10^9$/g [dry weight] for *B. distasonis*). *B. ovatus* was not detectable by our method. Since our lower limit of detection is approximately $1 \times 10^9$ to $2 \times 10^9$/g (dry weight) of feces, this is consistent with earlier estimates that indicated that the concentration of *B. ovatus* in feces is near or below this value. Thus, it appears that the ability to degrade a wide variety of polysaccharides is not the only major factor in determining which bacteria are the most numerous in the colon.**

Many species of bacteria that are found in the human colon require a fermentable carbohydrate for growth (2). Simple sugars and starches are efficiently absorbed before they reach the colon, but complex polysaccharides, which are not degraded appreciably in the stomach or small intestines and which reach the colon largely intact, may serve as important sources of carbon and energy for saccharolytic colon bacteria. The pool of carbohydrate that enters the colon is probably a constantly changing mixture, composed of both dietary polysaccharides (e.g., plant cell wall polysaccharides, glycoproteins from meat) and of host products (e.g., mucins, mucopolysaccharides from sloughed epithelial tissue). Given the high number of bacteria that reside in the colon, circa $10^{11}$/g (dry weight) of colon contents (3, 7, 8), competition for fermentable carbohydrate may be intense. If so, organisms that are able to utilize a wide variety of polysaccharides might have a competitive advantage for survival in the colon.

In two previous studies, Salyers et al. (11, 12) surveyed pure cultures representing the major species of colon bacteria for the ability to ferment polysaccharides. The results of these surveys indicated that most of the organisms that could degrade polysaccharides are members of the *Bacteroides*. Some species of *Bacteroides*, notably *Bacteroides thetaiotaomicron*, *B. ovatus*, and "*Bacteroides* group 3452-A" (an unnamed DNA homology group), could ferment not only host-derived mucopolysaccharides (e.g., hyaluronic acid, chondroitin sulfate) but also a wide variety of plant polysaccharides. Other species, such as *B. vulgatus* and *B. uniformis*, also fermented many plant polysaccharides but could not ferment mucopolysaccharides. Still other species, such as *B. distasonis* and *B. eggerthii*, could only ferment one or two plant polysaccharides. If the ability to utilize a variety of polysaccharides, particularly the ability to utilize host and plant polysaccharides, gives bacteria a competitive advantage in the colon, it might be expected that the more versatile polysaccharide-degrading species such as *B. thetaiotaomicron* or "*Bacteroides* group 3452-A" would be more numerous than *B. distasonis* or *B. uniformis*.

Moore and Holdeman, in their classic study of the species composition of human colonic flora (8), observed that members of the genus *Bacteroides* were among the most numerous bacteria isolated from feces, accounting for approximately 30% of all isolates. However, these organisms were phenotypically so similar that most *Bacteroides* isolates were identified as belonging to subspecies of *B. fragilis*. Not until subsequent DNA-DNA homology analyses were performed did it become evident that these subspecies differ significantly from one another at the genetic level (4). On this basis, many of the former *B. fragilis* subspecies were elevated to species rank, whereas some organisms were assigned to newly established species (1). As an example, some fecal isolates that Moore and Holdeman had originally identified as *B. fragilis* subsp. *thetaiotaomicron* ($2 \times 10^{10}$ to $3 \times 10^{10}$/g [dry weight] of feces) are now assigned to *B. thetaiotaomicron*, whereas others are assigned to *B. uniformis* based on DNA homology. Similarly, some fecal isolates that had been identified as *B. fragilis* subsp. *distasonis* ($1 \times 10^{10}$ to $2 \times 10^{10}$/g [dry weight] of feces) have now been assigned to *B. distasonis*, and others have been assigned to "*Bacteroides* group 3452-A" (an unnamed DNA homology group). There have been no subsequent attempts to determine the concentrations of these newly established species in feces because conventional methods for isolating, identifying, and enumerating colonic *Bacteroides* spp. are so cumbersome and time-consuming (2, 7). Moreover, these methods do not readily distinguish some of the new species (2, 4). Thus, it is unclear whether those species that have been identified as being among the most versatile polysaccharide-degrading bacteria (e.g., *B. thetaiotaomicron*, "*Bac-*

* Corresponding author.

TABLE 1. Hybridization of pBT-2 to pure cultures of different strains of *B. thetaiotaomicron*

| Strain no.[a] | % DNA homology with *B. thetaiotaomicron* type strain[b] | kcpm bound/$10^8$ disrupted bacteria[c] |
|---|---|---|
| 5482, Type strain | 100 | 4.2 |
| 0633 | 80 | 4.8 |
| 0940-1 | 91 | 4.0 |
| 2808-B | 93 | 4.1 |
| 3089 | 75 | 4.4 |
| 3164-A | 81 | 4.3 |
| 3443 | 76 | 4.0 |
| 5951 | 80 | 4.3 |
| 7330 | 86 | 4.1 |
| C11-15 | 92 | 3.8 |
| J19-34B | 87 | 3.9 |

[a] All strain numbers are Virginia Polytechnic Institute Anaerobe Laboratory designations.
[b] Data and source of strains from reference 4.
[c] kcpm bound to filter minus background. Each value is the average of quadruplicate determinations. Background was determined as the cpm bound to filters through which only media had been filtered. The average deviation was 0.3 kcpm.

*teroides* group 3452-A") are present in high concentrations in the colon, as would be expected if the ability to utilize a variety of polysaccharides provided a competitive advantage for bacteria in the colon.

Recently, we reported a method for estimating the concentrations of bacteria in feces by using species-specific DNA probes (5), and we used this method to enumerate *B. vulgatus* in feces. In the present report, we describe the use of DNA probes to enumerate *B. thetaiotaomicron*, *B. uniformis*, "*Bacteroides* group 3452-A", and *B. distasonis* in human fecal samples.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** The *Bacteroides* strains that were used in this study were obtained from the Anaerobe Laboratory of the Virginia Polytechnic Institute and State University, Blacksburg, Va. Unless otherwise noted, all strain numbers are Virginia Polytechnic Institute designations. In initial experiments, the specificity of each constructed DNA probe was tested against the following type strains: *B. vulgatus* 4245 (ATCC 8482), *B. thetaiotaomicron* 5482 (ATCC 29148), *B. ovatus* 0038 (ATCC 8483), *B. uniformis* 0061 (ATCC 8492), *B. fragilis* type I 2553 (ATCC 25285), *B. fragilis* type II 2393, *B. distasonis* 4243 (ATCC 8503), *Bacteroides* strain 3452-A (formerly a member of *B. fragilis* subsp. *distasonis*), *Bacteroides* strain B5-21 (formerly *B. fragilis* subsp. *A*), and *B. eggerthii* B8-51. Subsequently, a DNA probe was tested against different strains of the species for which it was specific; these strains are listed in Tables 1 through 4. The species designations for all *Bacteroides* strains cited in this report are based on DNA-DNA homology studies (4). For use in experiments, bacteria were grown at 37°C in a defined medium (10) with 0.5% glucose (wt/vol) as the carbon source, under an atmosphere of 80% nitrogen–20% carbon dioxide.

TABLE 2. Hybridization of pBU-2 to pure cultures of different strains of *B. uniformis*

| Strain no.[a] | % DNA homology with *B. uniformis* type strain[b] | kcpm bound/$10^8$ disrupted bacteria[c] |
|---|---|---|
| 0061, Type strain | 100 | 3.4 |
| 0909 | 82 | 3.6 |
| 3537 | 77 | 3.5 |
| C7-17 | 88 | 3.5 |
| C20-25 | 81 | 3.4 |
| R5-33 | 78 | 3.1 |
| T1-1 | 84 | 3.4 |

[a] See Table 1, footnote *a*.
[b] See Table 1, footnote *b*.
[c] kcpm bound to filter minus background. Each value is the average of quadruplicate determinations. Background was determined as the cpm bound to filters through which only media had been filtered. The average deviation was 0.25 kcpm.

TABLE 3. Hybridization of pBD-4 to pure cultures of different strains of *B. distasonis*

| Strain no.[a] | % DNA homology with *B. distasonis* type strain[b] | kcpm bound/$10^8$ disrupted bacteria[c] |
|---|---|---|
| 4243, Type strain | 100 | 2.0 |
| B1-20 | 81 | 1.9 |
| C18-7 | 80 | 2.3 |
| C19-17 | 91 | 2.2 |
| C30-45 | 91 | 2.1 |
| C1402 | 89 | 2.2 |
| S6A-50 | 88 | 2.2 |
| T3-25 | 88 | 2.0 |

[a] See TAble 1, footnote *a*.
[b] See Table 1, footnote *b*.
[c] kcpm bound to filter minus background. Each value is the average of quadruplicate determinations. Background was determined as the cpm bound to filters through which only media had been filtered. The average deviation was 0.2 kcpm.

**Construction and testing of species-specific DNA probes.** DNA probes that are specific for *B. uniformis*, *B. distasonis*, and "*Bacteroides* group 3452-A" were constructed by cloning random fragments of purified chromosomal DNA from each of these species in plasmid pBR322 according to the procedures previously reported for constructing DNA probes specific for *B. thetaiotaomicron* (6, 9). The specificity of the cloned DNA fragments was assessed by using the filtration and hybridization method that we have described earlier (5, 6, 9). In this method, the entire recombinant plasmid was first labeled with $^{32}P$ to a final specific activity of 15 to 25 μCi per microgram of DNA by standard nick-translation procedures (5, 9). The labeled plasmid was then incubated with nitrocellulose filters that contained DNA from the various *Bacteroides* species. Details of this procedure have been published previously (9). Hybridization of a $^{32}P$-labeled plasmid to the filtered DNA samples was measured by liquid scintillation counting. A plasmid probe was

TABLE 4. Hybridization of pB3-4 to pure cultures of different strains of "*Bacteroides* group 3452-A"

| Strain no.[a] | % DNA homology with *Bacteroides* 3452-A type strain[b] | kcpm bound/$10^8$ disrupted bacteria[c] |
|---|---|---|
| 3452-A, Type strain | 100 | 2.3 |
| 2308 | 90 | 2.1 |
| B6-11 | 93 | 2.4 |
| C7-8 | 90 | 2.6 |
| C10-2 | 88 | 2.2 |
| C14-3 | 92 | 2.2 |

[a] See Table 1, footnote *a*.
[b] See Table 1, footnote *b*.
[c] See Table 1, footnote *c*.

TABLE 5. Quantitation of *B. thetaiotaomicron* in human feces

| Amt of feces filtered (mg wet wt) | Amt of $^{32}$P-labeled pBT-2 bound (cpm)[a] in: Feces alone[d] Trial I | Feces alone[d] Trial II | Feces plus added bacteria[e] Trial I | Feces plus added bacteria[e] Trial II | Avg cpm/added $10^8$ bacteria[b] | % Pure culture[c] | Estimated concn (organisms/g of feces) ($\times 10^9$) Wet[f] | Dry[g] |
|---|---|---|---|---|---|---|---|---|
| Subject A | | | | | | | | |
| 5.0 | 135 | 121 | 341 | 409 | 1,235 | 29 | 2.2 | 8.8 |
| 7.5 | 193 | 174 | 613 | 578 | 1,370 | 33 | 1.9 | 7.6 |
| 10.0 | 303 | 208 | 768 | 827 | 1,354 | 32 | 2.0 | 8.0 |
| 12.5 | 289 | 303 | 828 | 1,012 | 1,148 | 27 | 2.2 | 8.8 |
| Subject B | | | | | | | | |
| 5.0 | 98 | 117 | 409 | 429 | 1,560 | 37 | 1.5 | 10.0 |
| 7.5 | 162 | 144 | 622 | 649 | 1,608 | 39 | 1.4 | 9.0 |
| 10.0 | 225 | 264 | 902 | 884 | 1,620 | 39 | 1.7 | 11.0 |
| 12.5 | 283 | 326 | 1,102 | 994 | 1,487 | 36 | 1.8 | 12.0 |

[a] Values reported are averages of duplicate determinations.
[b] [(cpm from feces + added bacteria) − cpm from feces alone]/number of added bacteria in the sample.
[c] Percentage of the cpm per $10^8$ bacteria obtained when a pure culture containing the same number of *B. thetaiotaomicron* was used.
[d] Trials I and II were performed in parallel on two different stool samples collected several days apart.
[e] A pure culture of *B. thetaiotaomicron* was added to the fecal suspension before processing, to a final concentration of $10^9$ added bacteria per ml, as an internal standard.
[f] *B. thetaiotaomicron* per g of wet feces = {cpm from feces/[(cpm from feces + added bacteria) − cpm from feces alone]} × $10^9$ added bacteria per ml/0.225 g of wet feces per ml.
[g] Based on average value for (fecal dry weight/fecal wet weight) of 25% for subject A and 15% for subject B.

considered specific for a species if the amount of hybridization (counts per minute [cpm]) detected between the probe and DNA from type strains of other species was 10% or less of that detected between the probe and DNA from the species from which the probe was derived. In general, probes had to be used within 4 weeks after being labeled with $^{32}$P to attain the sensitivity needed to detect fecal bacteria.

**Detection and enumeration of bacteria with species-specific DNA probes.** The procedure for using DNA probes to detect and enumerate bacteria in feces has been reported previously (5) and is described briefly below. For the current study, fecal specimens were obtained from two healthy adult volunteers. Feces were diluted and centrifuged to pellet bacteria. Bacteria were lysed with 0.5 N NaOH, and bacterial DNA was partially freed from protein and other contaminants by further centrifugation and by extraction with phenol-chloroform. Bacterial DNA was trapped on nitrocellulose paper by vacuum filtration, and the filters were then incubated with a $^{32}$P-labeled species-specific DNA probe. The amount of $^{32}$P-labeled probe that hybridized to DNA on the nitrocellulose was measured by liquid scintillation counting. To determine the concentration of a particular species in feces, we analyzed fecal samples to which known amounts of a pure culture of the given species had been added (as an internal standard) along with fecal samples to which no bacteria had been added. The amounts of feces that were filtered were in the range where (i) increasing amounts of filtered material resulted in a proportional increase in the amount of $^{32}$P-labeled probe bound to the filters and (ii) adding known amounts of a pure culture to the fecal sample

TABLE 6. Quantitation of *B. uniformis* in human feces

| Amt of feces filtered (mg wet wt) | Amt of $^{32}$P-labeled pBU-2 bound (cpm)[a] in: Feces alone[d] Trial I | Feces alone[d] Trial II | Feces plus added bacteria[e] Trial I | Feces plus added bacteria[e] Trial II | Avg cpm/added $10^8$ bacteria[b] | % Pure culture[c] | Estimated concn (organisms/g of feces) Wet[f] ($\times 10^9$) | Dry[g] ($\times 10^{10}$) |
|---|---|---|---|---|---|---|---|---|
| Subject A | | | | | | | | |
| 5.0 | 166 | 204 | 364 | 418 | 1,030 | 29 | 4.0 | 1.6 |
| 7.5 | 275 | 301 | 586 | 639 | 1,080 | 31 | 3.9 | 1.6 |
| 10.0 | 469 | 402 | 831 | 783 | 930 | 27 | 5.2 | 2.0 |
| 12.5 | 442 | 498 | 901 | 938 | 900 | 26 | 4.6 | 1.8 |
| Subject B | | | | | | | | |
| 5.0 | 107 | 133 | 342 | 398 | 1,250 | 36 | 2.1 | 1.4 |
| 7.5 | 173 | 157 | 547 | 575 | 1,320 | 39 | 1.9 | 1.2 |
| 10.0 | 265 | 239 | 769 | 747 | 1,265 | 37 | 2.2 | 1.5 |
| 12.5 | 331 | 349 | 1,929 | 1,967 | 1,215 | 35 | 2.5 | 1.7 |

[a] Values reported are averages of duplicate determinations.
[b] [(cpm from feces + added bacteria) − cpm from feces alone]/number of added bacteria in the sample.
[c] Percentage of the cpm per $10^8$ bacteria obtained when a pure culture containing the same number of *B. uniformis* was used.
[d] Trials I and II were performed in parallel on two different stool samples collected several days apart.
[e] A pure culture of *B. uniformis* was added to the fecal suspension before processing, to a final concentration of $10^9$ added bacteria per ml, as an internal standard.
[f] *B. uniformis* per g of wet feces = {cpm from feces/[(cpm from feces + added bacteria) − cpm from feces alone]} × $10^9$ added bacteria per ml/0.225 g of wet feces per ml.
[g] Based on an average value for (fecal dry weight/fecal wet weight) of 25% for subject A and 15% for subject B.

TABLE 7. Quantitation of *B. distasonis* in human feces

| Amt of feces filtered (mg wet wt) | Amt of $^{32}$P-labeled pBD-4 bound (cpm)[a] in: Feces alone[d] | | Feces plus added bacteria[e] | | Avg cpm/ added $10^8$ bacteria[b] | % Pure culture[c] | Estimated concn (organisms/g of feces) (× $10^9$) | |
|---|---|---|---|---|---|---|---|---|
| | Trial I | Trial II | Trial I | Trial II | | | Wet[f] | Dry[g] |
| Subject A | | | | | | | | |
| 7.5 | 60 | 81 | 186 | 220 | 442 | 22 | 2.1 | 8.4 |
| 10.0 | 71 | 82 | 291 | 258 | 495 | 25 | 1.5 | 6.2 |
| 12.5 | 95 | 119 | 354 | 318 | 458 | 23 | 1.9 | 7.5 |
| 15.0 | 116 | 127 | 367 | 392 | 430 | 21 | 1.8 | 7.2 |
| Subject B | | | | | | | | |
| 7.5 | 56 | 44 | 236 | 250 | 643 | 32 | 1.0 | 6.7 |
| 10.0 | 68 | 52 | 341 | 327 | 685 | 33 | 0.8 | 5.8 |
| 12.5 | 71 | 85 | 421 | 405 | 670 | 34 | 0.9 | 6.2 |
| 15.0 | 89 | 113 | 463 | 497 | 631 | 30 | 1.1 | 7.1 |

[a] Values reported are averages of duplicate determinations.
[b] [(cpm from feces + added bacteria) − cpm from feces alone]/number of added bacteria in the sample.
[c] Percentage of the cpm per $10^8$ bacteria obtained when a pure culture containing the same number of *B. distasonis* was used.
[d] Trials I and II were performed in parallel on two different stool samples collected several days apart.
[e] A pure culture of *B. distasonis* was added to the fecal suspension before processing, to a final concentration of $10^9$ added bacteria per ml, as an internal standard.
[f] *B. distasonis* per g of wet feces = {cpm from feces/[(cpm from feces + added bacteria) − cpm from feces alone]} × $10^9$ added bacteria per ml/0.25 g of wet feces per ml.
[g] Based on an average value for (fecal dry weight/fecal wet weight) of 25% for subject A and 15% for subject B.

before processing also resulted in a proportionate increase in the amount of $^{32}$P-labeled probe bound to the filters.

## RESULTS

**Specificity of DNA probes.** By screening recombinant plasmids for hybridization to the DNA from type strains of various *Bacteroides* species, we identified DNA probes that were specific for *B. uniformis*, *B. distasonis*, and "*Bacteroides* group 3452-A". pBU-2, the probe that hybridized to the DNA from *B. uniformis* but not to DNA from any other *Bacteroides* species tested, contained a 2.8-kilobase fragment of chromosomal DNA from *B. uniformis* 0061. Similarly, pBD-4 hybridized specifically to DNA from *B. distasonis*, and pB3-4 hybridized specifically to the DNA from "*Bacteroides* group 3452-A"; these recombinant plasmids contained, respectively, a 2.8-kilobase fragment of DNA from *B. distasonis* 4243 and a 1.1-kilobase fragment of DNA from "*Bacteroides* group 3452-A". The characteristics of pBT-2, a probe which is specific for *B. thetaiotaomicron*, have been described previously (9).

**Quantitation of bacteria with DNA probes.** The amount of species-specific, $^{32}$P-labeled DNA probe which hybridized to the DNA that was trapped on a nitrocellulose filter when a lysed pure culture of *B. thetaiotaomicron*, *B. uniformis*, *B. distasonis*, or "*Bacteroides* group 3452-A" had been filtered was proportional to the number of bacteria in the sample, within a range of approximately $10^7$ to $10^8$ bacteria. These results were similar to those obtained previously for *B. vulgatus* (5). The slope of the hybridization curves obtained with a given probe (i.e., kcpm bound per $10^8$ lysed bacteria

TABLE 8. Quantitation of "*Bacteroides* group 3452-A" in human feces

| Amt of feces filtered (mg wet wt) | Amt of $^{32}$P-labeled pB3-4 bound (cpm)[a] in: Feces alone[d] | | Feces plus added bacteria[e] | | Avg cpm/ added $10^8$ bacteria[b] | % Pure culture[c] | Estimated concn (organisms/g of feces) (× $10^9$) | |
|---|---|---|---|---|---|---|---|---|
| | Trial I | Trial II | Trial I | Trial II | | | Wet[f] | Dry[g] |
| Subject A | | | | | | | | |
| 7.5 | 24 | 37 | 201 | 189 | 494 | 21 | 0.74 | 2.9 |
| 10.0 | 62 | 51 | 257 | 265 | 511 | 22 | 1.1 | 4.4 |
| 12.5 | 64 | 70 | 365 | 333 | 560 | 25 | 0.96 | 3.8 |
| 12.5 | 84 | 77 | 370 | 392 | 501 | 21 | 1.0 | 4.0 |
| Subject B | | | | | | | | |
| 7.5 | 26 | 37 | 206 | 219 | 603 | 27 | 0.7 | 4.6 |
| 10.0 | 67 | 51 | 313 | 285 | 602 | 27 | 1.0 | 6.4 |
| 12.5 | 79 | 67 | 390 | 418 | 662 | 29 | 0.8 | 5.8 |
| 15.0 | 91 | 81 | 463 | 435 | 605 | 27 | 0.9 | 6.3 |

[a] Values reported are averages of duplicate determinations.
[b] [(cpm from feces + added bacteria) − cpm from feces alone]/number of added bacteria in the sample.
[c] Percentage of the cpm per $10^8$ bacteria obtained when a pure culture containing the same number of "*Bacteroides* group 3452-A" was used.
[d] Trials I and II were performed in parallel on two different stool samples collected several days apart.
[e] A pure culture of "*Bacteroides* group 3452-A" was added to the fecal suspension before processing, to a final concentration of $10^9$ added bacteria per ml, as an internal standard.
[f] "*Bacteroides* group 3452-A" per g of wet feces = {cpm from feces/[(cpm from feces + added bacteria) − cpm from feces alone]} × $10^9$ added bacteria per ml/0.25 g of wet feces per ml.
[g] Based on an average value for (fecal dry weight/fecal wet weight) of 25% for subject A and 15% for subject B.

TABLE 9. Comparison of concentrations of *Bacteroides* spp. in human feces obtained by different methods

| Former species designation | Concn of bacteria by conventional methods[a] (per g of dry wt) | Current species designation[b] | Concn of bacteria by DNA probe method (per g of dry wt) |
|---|---|---|---|
| *B. fragilis* subsp. *vulgatus* | $3.0 \times 10^{10}$–$6.0 \times 10^{10}$ | *B. vulgatus* | $2.0 \times 10^{10}$–$3.0 \times 10^{10}$ |
| *B. fragilis* subsp. *thetaiotaomicron* | $2.0 \times 10^{10}$–$3.0 \times 10^{10}$ | *B. thetaiotaomicron* | $0.8 \times 10^{10}$–$1.2 \times 10^{10}$ |
| | | *B. uniformis* | $1.2 \times 10^{10}$–$2.0 \times 10^{10}$ |
| *B. fragilis* subsp. *distasonis* | $1.0 \times 10^{10}$–$2.0 \times 10^{10}$ | *B. distasonis* | $5.8 \times 10^{9}$–$8.4 \times 10^{9}$ |
| | | "*Bacteroides* group 3452-A" | $3.0 \times 10^{9}$–$6.0 \times 10^{9}$ |
| *B. fragilis* subsp. *ovatus* | $0.3 \times 10^{9}$–$3.0 \times 10^{9}$ | *B. ovatus* | Not detectable |

[a] Concentrations determined previously by Moore and Holdeman (3, 8) by using conventional plating techniques and biochemical tests for identification of colonic *Bacteroides* spp.

[b] New species designations based on DNA-DNA homology studies (4).

filtered) was similar for all strains of the species for which the probe was specific (Tables 1 through 4). There was no detectable hybridization between $^{32}$P-labeled pBR322 and the DNA from any *Bacteroides* species tested.

**Enumerating bacteria in feces.** The results of measurements of the concentrations of *B. thetaiotaomicron*, *B. uniformis*, *B. distasonis*, and "*Bacteroides* group 3452-A" in two different fecal specimens from each of two subjects are shown in Tables 5 through 9. In general, as in our previous study (5), we used two different concentrations of added bacteria to generate the standard curve, although for simplicity only one is shown in Tables 5 through 8. For example, for *B. thetaiotaomicron* and *B. uniformis* (Tables 5 and 6), reducing the number of added bacteria by half gave a proportionate decrease in the amount of probe that was bound to the filter (data not shown). With *B. distasonis* and "*Bacteroides* group 3452-A", however, it was not possible to generate a multipoint linear standard curve. The increase in cpm observed when $10^9$ bacteria per ml was added was too low to make it feasible to use $5 \times 10^8$ bacteria per ml. When $2 \times 10^9$ bacteria per ml was added, there was an increase in bound cpm, but the increase was about half that seen when $10^9$ bacteria per ml was added. Moreover, when concentrations of feces higher than 12 to 15 mg of equivalent fecal wet weight were filtered, the increase in bound cpm obtained by adding $10^9$ bacteria per ml was lower than that observed at lower concentrations of feces. These results indicate that we were close to the capacity of the nitrocellulose filter. Because of this, the concentration values obtained for *B. distasonis* and "*Bacteroides* group 3452-A" could be overestimates. Since, however, the percent recovery is about the same as that for *B. thetaiotaomicron* and *B. uniformis*, the estimate is probably a reasonable one. The data in Tables 7 and 8 shows that the concentration of *B. distasonis* is approximately the same as that of "*Bacteroides* group 3452-A".

The values that were obtained for different fecal specimens from the same subject (trials I and II) generally varied by less than 15%. The results that were obtained from the two subjects were also similar. In the feces of either subject, the concentrations of *B. thetaiotaomicron* ($7.6 \times 10^9$ to $8.8 \times 10^9$ per gram of dry feces for subject A, $9.0 \times 10^9$ to $12.0 \times 10^9$ per gram of dry feces for subject B) were somewhat lower than the concentrations of *B. uniformis* ($1.6 \times 10^{10}$ to $2.0 \times 10^{10}$ per gram of dry feces for subject A, $1.2 \times 10^{10}$ to $1.7 \times 10^{10}$ per gram of dry feces for subject B), but the concentrations were of the same order of magnitude (Tables 5 and 6). Similarly, the concentrations of "*Bacteroides* group 3452-A" ($2.9 \times 10^9$ to $40 \times 10^9$ per gram of dry feces for subject A, $4.6 \times 10^9$ to $6.3 \times 10^9$ per gram of dry feces for subject B) appeared somewhat lower than the concentrations of *B. distasonis* ($6.2 \times 10^9$ to $8.4 \times 10^9$ per gram of dry feces for subject A, $5.8 \times 10^9$ to $7.1 \times 10^9$ per gram of dry feces for subject B; Tables 7 and 8). For all concentrations of feces that were analyzed in this study, no hybridization of $^{32}$P-labeled pBR322 to the DNA on the nitrocellulose filters was detected.

## DISCUSSION

The results of this study, taken together with the results of a previous study in which we used a DNA probe to enumerate *B. vulgatus* in the feces of subject A (5), are in general agreement with estimates that were obtained previously by Moore and Holdeman (8), who used conventional bacteriological methods (Table 9). That is, the sum of the concentrations that we obtained for *B. thetaiotaomicron* and *B. uniformis* is comparable to the concentration reported previously for *B. fragilis* subsp. *thetaiotaomicron*. Similarly, the sum of concentrations of *B. distasonis* and "*Bacteroides* group 3452-A" is comparable to that reported previously for *B. fragilis* subsp. *distasonis*. By using species-specific DNA probes, which allowed us to detect and enumerate species that are not readily distinguished by biochemical tests, we were able to show that *B. thetaiotaomicron*, *B. uniformis*, *B. distasonis*, and "*Bacteroides* group 3452-A" are all present in high concentrations in feces. We also attempted to determine the concentration of *B. ovatus* (formerly *B. fragilis* subsp. *ovatus*) in feces by using a species-specific DNA probe, but we were unable to detect this organism (data not shown). Given that the lower limit of detection for our method appears to be approximately $1 \times 10^9$ to $2 \times 10^9$ bacteria per gram of dry feces (cf. Tables 7 and 8), our inability to detect *B. ovatus* is consistent with the earlier estimates of Moore and Holdeman (3, 8) which indicated that the concentration of *B. fragilis* subsp. *ovatus* in feces was near or below this level. The concentrations of other *Bacteroides* species, such as *B. eggerthii* (formerly *B. fragilis* subsp. *eggerthii*) and *B. fragilis* (formerly *B. fragilis* subsp. *fragilis*), which have also been estimated to be near this level (3, 8), would probably not be detectable by our method.

By allowing us to obtain concentrations for those *Bacteroides* species for which estimates have not previously been possible, our method also permitted us to test the hypothesis that the ability to degrade a variety of polysaccharides is a major factor in determining which species are most numerous in the colon. Although our results indicate that the more versatile polysaccharide-degrading *Bacteroides* species (*B. thetaiotaomicron* and "*Bacteroides* group 3452-A") do in fact appear to be present in high concentrations, these species do not appear to be more numerous than closely

related, but less versatile, *Bacteroides* species (*B. vulgatus*, *B. uniformis*, *B. distasonis*). Thus, factors other than versatility in polysaccharide catabolism are probably also important for the survival of organisms in the colon.

An alternative explanation is that the collection of polysaccharides that was used by Salyers et al. (10, 11) to assess polysaccharide utilization by the major species of colon bacteria may not have been complete enough and may not have accurately represented all the types of substrates that are available for organisms in the colon because this survey was limited to polysaccharides that were commercially available. Moreover, the polysaccharides that were used in the survey were soluble, purified polysaccharides, whereas in nature these compounds frequently occur in complex aggregates such as plant cell walls. It is also possible that some colon bacteria can degrade polysaccharides only in cooperation with other organisms. Such phenomena would not be observed in studies that use pure cultures.

The two volunteers whose feces were analyzed in this study were healthy adult males who consumed a typical Western diet. Subject A admitted that he generally included high amounts of fiber in his diet in the form of several servings of fruit, vegetables, and grain daily. This may account for the finding that fecal dry weight was higher for subject A (averaging 25% of wet weight) than for subject B (averaging 15%). This may also explain the finding that although equivalent amounts of wet feces were assayed from both subjects, the amount of internal standard that could be detected when bacteria were added to the feces of subject A was consistently lower than the amount detected when the same number of bacteria was added to the feces of subject B (cf. column 7, Tables 5 through 8). These findings underscore the necessity of using an internal standard for every fecal sample to be analyzed.

The requirement for an internal standard not only makes the type of analysis presented here more cumbersome but also places limits on the concentration of organisms that can be quantitated with confidence. In the case of the more numerous species such as *B. vulgatus*, *B. uniformis* or *B. thetaiotaomicron*, a standard curve consisting of two or more points could be generated. The limiting factor is the binding capacity of the nitrocellulose filter. In the case of less numerous species, such as *B. distasonis* and "*Bacteroides* group 3452-A", the concentration of organisms was low enough compared with other organisms in the specimen that we were unable to generate a multipoint linear standard curve. Accordingly, the concentrations given in Table 9 had to be computed from a single concentration of added bacteria and could be an overestimate. Nonetheless, our results show that these species are present in approximately equal numbers. Thus, our procedure can provide information about relative concentrations of species even when the concentrations are too close to the limit of detection to be quantitated accurately.

## ACKNOWLEDGMENTS

We thank Robert E. McCarthy for stimulating discussions and helpful suggestions concerning this work. We acknowledge the excellent clerical assistance of Sharon Kirk.

This work was supported by Public Health Service grant AI-17876 from the National Institutes of Health.

## LITERATURE CITED

1. **Cato, E. P., and J. L. Johnson.** 1976. Reinstatement of species rank for *Bacteroides fragilis*, *B. ovatus*, *B. distasonis*, *B. thetaiotaomicron*, and *B. vulgatus*: designation of neotype strains for *Bacteroides fragilis* (Veillon and Zuber) Castellani and Chalmers and *Bacteroides thetaiotaomicron* (Distaso) Castellani and Chalmers. Int. J. Syst. Bacteriol. **26**:230–237.
2. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
3. **Holdeman, L. V., I. J. Good, and W. E. C. Moore.** 1976. Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. Appl. Environ. Microbiol. **31**:359–375.
4. **Johnson, J. L.** 1978. Taxonomy of the bacteroides. I. Deoxyribonucleic acid homologies among *Bacteroides fragilis* and other saccharolytic *Bacteroides* species. Int. J. Syst. Bacteriol. **28**:245–256.
5. **Kuritza, A. P., and A. A. Salyers.** 1985. Use of a species-specific DNA hybridization probe for enumerating *Bacteroides vulgatus* in human feces. Appl. Environ. Microbiol. **50**:958–964.
6. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Moore, W. E. C., E. P. Cato, and L. V. Holdeman.** 1978. Some current concepts in intestinal bacteriology. Am. J. Clin. Nutr. **31**:S33–S42.
8. **Moore, W. E. C., and L. V. Holdeman.** 1974. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. Appl. Microbiol. **27**:961–979.
9. **Salyers, A. A., S. P. Lynn, and J. F. Gardner.** 1983. Use of randomly cloned DNA fragments for identification of *Bacteroides thetaiotaomicron*. J. Bacteriol. **154**:287–293.
10. **Salyers, A. A., and M. O'Brien.** 1980. Cellular location of enzymes involved in chondroitin sulfate breakdown by *Bacteroides thetaiotaomicron*. J. Bacteriol. **143**:772–780.
11. **Salyers, A. A., J. R. Vercellotti, S. E. H. West, and T. D. Wilkins.** 1977. Fermentation of mucin and plant polysaccharides by strains of *Bacteroides* from the human colon. Appl. Environ. Microbiol. **33**:319–322.
12. **Salyers, A. A., S. E. H. West, J. R. Vercellotti, and T. D. Wilkins.** 1977. Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the human colon. Appl. Environ. Microbiol. **34**:529–533.