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PCR Detection and Quantitation of Predominant Anaerobic Bacteria in Human and Animal Fecal Samples

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PCR procedures based on 16S rRNA gene sequences specific for 12 anaerobic bacteria that predominate in the human intestinal tract were developed and used for quantitative detection of these species in human (adult and baby) feces and animal (rat, mouse, cat, dog, monkey, and rabbit) feces. *Fusobacterium prausnitzii*, *Peptostreptococcus productus*, and *Clostridium clostridiiforme* had high PCR titers (the maximum dilutions for positive PCR results ranged from 10^{-3} to 10^{-8}) in all of the human and animal fecal samples tested. *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, and *Eubacterium limosum* also showed higher PCR titers (10^{-2} to 10^{-6}) in adult human feces. The other bacteria tested, including *Escherichia coli*, *Bifidobacterium adolescentis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Eubacterium biforme*, and *Bacteroides distasonis*, were either at low PCR titers (less than 10^{-2}) or not detected by PCR. The reported PCR procedure including the fecal sample preparation method is simplified and rapid and eliminates the DNA isolation steps.

The microflora in the human intestinal tract is an extremely complex yet relatively stable ecological community that is populated by an excess of 10^{11} bacterial cells per g of content and contains more than 400 bacterial species in the colon (9), the predominant genera being *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Clostridium*, *Fusobacterium*, *Ruminococcus*, *Peptococcus*, and *Peptostreptococcus* (9). Indigenous intestinal microorganisms play several significant roles in human health because they aid in the digestion of food, metabolize drugs and foreign compounds, produce essential vitamins, and help prevent pathogens from colonizing the gastrointestinal tract (6–8, 10, 14). The intestinal microbes have also been implicated in causing colon cancer (2, 12, 16). Because of the importance of these bacteria, studies are being conducted to further elucidate the role of these organisms in human physiology and to determine factors which affect their populations in the gastrointestinal tract. In order to effectively study such a complex community, it is necessary to have quick and accurate methods for enumerating the various populations. In addition, the development of rapid, specific indicators for the presence of human intestinal bacteria would be a major asset for monitoring environmental samples for fecal contamination (3, 11, 13, 17).

The traditional methods for identifying fecal bacteria include various culture techniques, bacteriological isolations, biochemical tests, morphological examination, and analysis of volatile and nonvolatile fatty acid production. More recently, gas-liquid chromatography of bacterial cellular fatty acids has been used to identify the species. These methods are extremely labor-intensive and time-consuming. In addition, sometimes they cannot distinguish the bacteria on the species level (3, 13, 17, 27).

In contrast, PCR allows the rapid and specific detection of a wide range of bacterial species and it has become a key procedure for detecting microorganisms (5). Previously, we have used PCR to monitor *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, *Bacteroides distasonis*, *Clostridium clostridiiforme*, *Clostridium perfringens*, and *Clostridium leptum* in an in vitro semicontinuous culture system designed to mimic the human gastrointestinal tract (20). Recently, Kreader (13) also reported PCR-hybridization assays for the detection of *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, and *Bacteroides distasonis* in human and animal fecal samples. The purpose of this study was to develop PCR methods for detecting more bacterial species that are numerically predominant in fecal samples. The choice of bacteria, including *Fusobacterium prausnitzii*, *Eubacterium biforme*, *Eubacterium limosum*, *Peptostreptococcus productus*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Lactobacillus acidophilus*, and *Escherichia coli*, was based on the reports of Moore and coworkers (15, 16) on the relative frequency of bacterial species in the normal fecal flora and on other microbial studies of the human gastrointestinal tract (9). The effectiveness of PCR methods specific for 12 bacterial species was tested by applying the procedures to fecal samples. It is anticipated that these results will provide a valuable tool for gathering more comprehensive quantitative and qualitative information in any microbial study of the gastrointestinal tract.

MATERIALS AND METHODS

Bacterial strains, culture medium, and growth conditions. A complete list of bacterial strains used in this study is given in Table 1. Anaerobic bacteria were cultured at 37°C either in prereduced anaerobically sterilized brain heart infusion broth supplemented with vitamin K and hemin (BHI; Carr-Scarborough Microbiologicals, Stone Mountain, Ga.), inoculated under an oxygen-free cannula under 85% nitrogen, 10% hydrogen, and 5% carbon dioxide, or on prereduced anaerobically sterilized brucella-blood agar plates supplemented with vitamin K and hemin (Anaerobic Systems, San Jose, Calif.), inoculated in an anaerobic gas chamber (Coy Laboratory Products, Inc., Ann Arbor, Mich.) under 85% N_2, 10% H_2, and 5% CO_2. Culture dilutions were made under an oxygen-free gas cannula in dilution blanks (12a). Aerobic and facultative bacteria were cultured aerobically in tryptic soy broth–0.5% yeast extract (TSBYE) medium (Difco Laboratories, Detroit, Mich.) at 37°C overnight.

PCR primers. All primer sets were designed from the 16S rRNA gene sequences available from GenBank, except that the primer set for *E. coli* was modified from that of Candrian et al. (4) and the target sequence was the *E. coli malB* promoter gene. The 16S rRNA gene-based primers were chosen from the variable regions by the computer program ALIGN (Scientific & Educational Software, State Line, Pa.). The GenBank program BLAST (1) was used to ensure that the proposed primers were complementary with the target species but not with other species. Table 2 lists all of the 12 sets of primers used in this study. All of the primers were compared with 657,579 sequences in the GenBank, and none were found to have the exact same sequence as the nontargeted sequence. However, all of the primers were found to have the same sequence as or a sequence complementary to the target sequence. A few primers were found

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TABLE 1. Bacterial strains assayed by PCR in this study

Bacterium[a]	Strain	PCR result[b]							
		BIA	BIL	EBI	ELI	FPR	PSP	LAA	ECO
Bifidobacterium adolescentis	ATCC 15703	+	−	−	−	−	−	−	−
Bifidobacterium longum	ATCC 15707	−	+	−	−	−	−	−	−
Bifidobacterium infantis	ATCC 15697	−	−						
Eubacterium biforme	ATCC 27806			+	−		−		
Eubacterium limosum	ATCC 8486			−	+		−		
Eubacterium aerofaciens	ATCC 25986			−	−	−	−		−
Eubacterium lentum	ATCC 25559	−	−	−	−	−	−	−	−
Eubacterium hadrum	ATCC 29173	−	−	−	−	−	−	−	−
Eubacterium siracum	ATCC 29066	−	−	−	−	−	−	−	−
Fusobacterium prausnitzii	ATCC 27768					+			
Fusobacterium prausnitzii	ATCC 27766					+			
Fusobacterium nucleatum	ATCC 25586	−	−	−	−	−	−	−	−
Fusobacterium mortiferum	ATCC 9817	−				−			
Peptostreptococcus productus	ATCC 27340	−	−	−	−	−	+	−	−
Peptostreptococcus asaccharolyticus	ATCC 29743						−		
Peptostreptococcus anaerobius	ATCC 27337						−		
Peptostreptococcus magnus	ATCC 14955						−		
Lactobacillus acidophilus	ATCC 332	−	−	−	−	−	−	+	−
Propionibacterium acnes	ATCC 6919	−	−	−	−	−	−	−	−
Escherichia coli	XL-1								+
Escherichia coli	ATCC 25922	−	−	−	−	−	−	−	+
Escherichia coli (O78:H11)	ATCC 35401								+
Escherichia coli	DH5α								+
Escherichia coli	HB101								+
Shigella flexneri	ATCC 12022	−	−	−	−	−	−	−	+
Yersinia enterocolitica	ATCC 27729	−	−	−	−	−	−	−	−
Bacteroides distasonis	ATCC 8503	−	−	−	−	−	−	−	−
Bacteroides thetaiotaomicron	ATCC 29148	−	−	−	−	−	−	−	−
Bacteroides vulgatus	ATCC 8482	−	−	−	−	−	−	−	−
Clostridium clostridiiforme	ATCC 25537	−	−	−	−	−	−	−	−
Salmonella typhimurium	ATCC 14028	−	−	−	−	−	−	−	−

[a] In addition to the bacterial species listed in the table, we also obtained negative PCR results for all of the eight PCR primer sets for the following bacterial species: *Actinomyces naeslundii* ATCC 12104, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Citrobacter freundii* ATCC 25405, *Enterococcus faecalis* ATCC 19433, *Corynebacterium kutscheri* ATCC 15677, *Bacillus subtilis* ATCC 6051, *Staphylococcus aureus* ATCC 25923, *Streptococcus equi* ATCC 9528, *Proteus mirabilis* ATCC 7002, *Listeria monocytogenes* ATCC 15313, *Campylobacter jejuni* ATCC 35918, *Mycoplasma gallisepticum* ATCC 5969, *Micrococcus luteus* ATCC 4698, *Carnobacterium piscicola* ATCC 3586, *Vibrio vulnificus* ATCC 27562, and *Pasteurella pneumotropica* ATCC 35149.

[b] +, positive; −, negative; blank, not determined. Results of the PCR specificity test for the *Bacteroides* spp. and *Clostridium* sp. were reported previously (19). Abbreviations for bacteria: BIA, *Bifidobacterium adolescentis*; BIL, *Bifidobacterium longum*; EBI, *Eubacterium biforme*; ELI, *Eubacterium limosum*; FPR, *F. prausnitzii*; PSP, *P. productus*; LAA, *L. acidophilus*; ECO, *E. coli.*

to have only one or two mismatches to the nontargeted sequence, but the second primer in the primer set was found to be very different from the same nontargeted sequence.

The oligonucleotide primers, synthesized by National Biosciences, Inc. (Plymouth, Minn.), were unpurified grade.

Fecal sample preparation and PCR amplification procedure. Fresh feces were collected from healthy humans or animals (rats, mice, cats, a dog, a monkey, and a rabbit) and analyzed by PCR within 1 to 4 h of collection. A 1-g (wet weight) feces specimen was added to 9 ml of sterile phosphate-buffered saline (PBS; 0.05 M, pH 7.4) and mixed by inverting and vortexing the tube for 5 to 10 min. The samples were then centrifuged at low speed (200 × *g*) for 5 min to collect the upper phases. This centrifugation step was repeated three times. The upper phases were then centrifuged at 9,000 × *g* for 3 min to collect the bacterial cells in the pellets. The cells in the pellets were washed four times with PBS and once with water, resuspended in 0.1 ml of distilled water, serially diluted in 0.1 ml of 1% Triton X-100, heated at 100°C for 5 min, and immediately cooled in ice-water. Cells in pure cultures were directly centrifuged at 9,000 × *g* and then were washed twice with PBS and once with water, resuspended, diluted, boiled, and cooled in ice-water as described above. PCR tests were performed without isolation of the DNA; i.e., 2 μl of each sample was directly added to 23 μl of PCR mixture containing 50 mM Tris-HCl (pH 8.5), 20 mM KCl, 3 mM $MgCl_2$, 0.05% bovine serum albumin (catalog no. A-4378; Sigma Chemical Co., St. Louis, Mo.), 0.25 mM each deoxynucleoside triphosphate (dATP, dTTP, dCTP, and dGTP), 0.25 μM each primer, and 0.9 U of *Taq* polymerase. The PCR was conducted in a 1605 Air-Thermal Cycler (Idaho Technology, Idaho Falls, Idaho). The amplification conditions were one cycle of 94°C for 15 s and then 35 cycles of 94°C for 3 s, 50°C for 10 s, and 74°C for 35 s at the transition speed S-9, and finally one cycle of 74°C for 2 min and 45°C for 2 s. The PCR products (6 to 10 μl of each) were separated by electrophoresis in agarose gels containing ethidium bromide (1 μg/ml).

The direct microscopic method (with a Petroff-Hausser counting chamber and a phase-contrast microscope) used for determining the PCR sensitivity was previously described (21).

TABLE 2. PCR primers used in this study[a]

Bacterium	Primer	Sequence (5′→3′)	PCR product size (bp)
Bifidobacterium adolescentis	BIA-1	GGAAAGATTCTATCGGTATGG	244
	BIA-2	CTCCCAGTCAAAAGCGGTT	
Bifidobacterium longum	BIL-1	GTTCCCGACGGTCGTAGAG	153
	BIL-2	GTGAGTTCCCGGCATAATCC	
Eubacterium biforme	EBI-1	GCTAAGGCCATGAACATGGA	463
	EBI-2	GCCGTCCTCTTCTGTTCTC	
Eubacterium limosum	ELI-1	GGCTTGCTGGACAAATACTG	274
	ELI-2	CTAGGCTCGTCAGAAGGATG	
Fusobacterium prausnitzii	FPR-1	AGATGGCCTCGCGTCCGA	199
	FPR-2	CCGAAGACCTTCTTCCTCC	
Peptostreptococcus productus	PSP-1	AACTCCGGTGGTATCAGATG	268
	PSP-2	GGGGCTTCTGAGTCAGGTA	
Lactobacillus acidophilus	LAA-1	CATCCAGTGCAAACCTAAGAG	286
	LAA-2	GATCCGCTTGCCTTCGCA	
Escherichia coli	ECO-1	GACCTCGGTTTAGTTCACAGA	585
	ECO-2	CACACGCTGACGCTGACCA	
Bacteroides thetaiotaomicron	BT-1	GGCAGCATTTCAGTTTGCTTG	423
	BT-2	GGTACATACAAAATTCCACACGT	
Bacteroides vulgatus	BV-1	GCATCATGAGTCCGCATGTTC	287
	BV-2	TCCATACCCGACTTTATTCCTT	
Bacteroides distasonis	BD-1	GTCGGACTAATACCGCATGAA	273
	BD-2	TTACGATCCATAGAACCTTCAT	
Clostridium clostridiiforme	CC-1	CCGCATGGCAGTGTGTGAAA	255
	CC-2	CTGCTGATAGAGCTTTACATA	

[a] All primer sets were designed from the 16S rRNA gene sequences available from GenBank, except that the primer set for *E. coli* was modified from that of Candrian et al. (4) and the target sequence was the *E. coli malB* promoter gene.

RESULTS

Development of PCR procedures. PCR methods for detecting and quantifying 12 of the bacterial species that predominate in the human gastrointestinal tract were developed. The specificities of the PCR methods were tested, and the results are listed in Table 1. PCR specificities for the *Bacteroides* spp. and *Clostridium* spp. were reported previously (20). The PCR procedures were highly specific for the target species, except for *E. coli*, which cross-reacted with *Shigella flexneri* (Table 1). The *E. coli* primer set was modified from that of Candrian et al. (4) by deleting 4 to 6 bases in the 5′ end to fit our same PCR conditions. Candrian et al. reported that the PCR primer set was positive for all 27 *E. coli* strains tested but was negative with the other species tested (4). However, they did not test for *Shigella* spp. Deletions from the 5′ end of the primers did not affect the PCR specificity. We have tested five more strains of *E. coli* and four *Shigella* spp., all of which gave positive results (data not shown).

Figure 1 shows the PCR products in an agarose gel for each of the 12 bacteria tested with their respective primers, which are given in Table 2.

PCR sensitivities for all of the 12 PCR procedures were determined with pure cultures of the 12 species. The results for one species, *Bifidobacterium longum* ATCC 15707, are given in Fig. 2. As few as two cells of *Bifidobacterium longum* were detected by this PCR method (Fig. 2, lane 6). The PCR sensitivities for other species varied and were dependent upon the species. The PCR sensitivities were 4 cells for *F. prausnitzii*, 40 cells for *P. productus*, 10,000 cells for *Bifidobacterium adolescentis*, 100 cells for *L. acidophilus*, 1,000 cells for *Eubacterium biforme*, 1,000 cells for *Eubacterium limosum*, and 20 cells for *E. coli* (data not shown). The PCR sensitivities for *Bacteroides vulgatus*, *Bacteroides thetaiotaomicron*, *Bacteroides distasonis*, and *C. clostridiiforme* were two to four cells as reported previously (20).

Use of PCR procedures for quantitative detection of 12 bacterial species in human and animal feces. Fecal samples from seven adult humans, two baby humans, two mice (BALB/c), two rats (Fischer), two cats, one dog, one monkey (rhesus), and one rabbit (New Zealand) were examined by the 12 PCR methods. Figure 3 illustrates the PCR titers of each species in adult human feces. Table 3 shows the results for all fecal samples tested. *F. prausnitzii*, *P. productus*, and *C. clostridiiforme* had high PCR titers (the maximum dilutions for positive PCR results) in all of the human and animal fecal samples tested. The PCR titers for these three species were 10^{-3} to

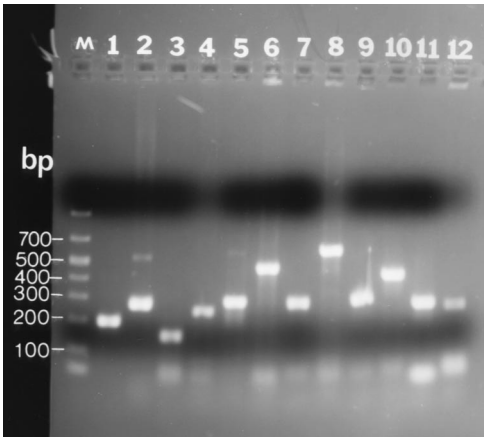

FIG. 1. PCR products in a 2% agarose gel for the 12 bacterial species tested with their respective primers. Lanes: M, DNA size markers (sizes are shown at the left); 1, *F. prausnitzii* (199 bp); 2, *P. productus* (268 bp); 3, *Bifidobacterium longum* (153 bp); 4, *Bifidobacterium adolescentis* (244 bp); 5, *L. acidophilus* (286 bp); 6, *Eubacterium biforme* (463 bp); 7, *Eubacterium limosum* (276 bp); 8, *E. coli* (585 bp); 9, *Bacteroides vulgatus* (287 bp); 10, *Bacteroides thetaiotaomicron* (423 bp); 11, *Bacteroides distasonis* (273 bp); 12, *C. clostridiiforme* (255 bp).

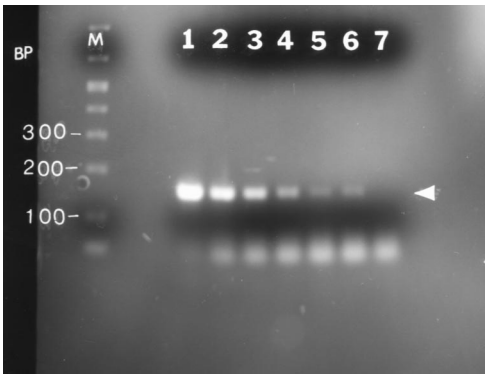

FIG. 2. PCR sensitivity test for pure culture of *Bifidobacterium longum* ATCC 15707. PCR products (153 bp) were separated in a 2% agarose gel containing ethidium bromide. Lane M contains DNA size markers (sizes are shown at the left). Numbered lanes contain the following amounts of *Bifidobacterium longum*: lane 1, 10^4 cells; lane 2, 10^3 cells; lane 3, 10^2 cells; lane 4, 10 cells; lane 5, 4 cells; lane 6, 2 cells; lane 7, 0 cells (negative control).

10^{-8}. *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, and *Eubacterium limosum* also showed higher PCR titers (10^{-2} to 10^{-6}) in adult human feces. The other six species either were at low PCR titers (less than 10^{-2}) or were not detected by the PCR. Baby human feces contained almost no *Bacteroides* cells but contained high numbers of *Bifidobacterium* cells compared with the other fecal samples.

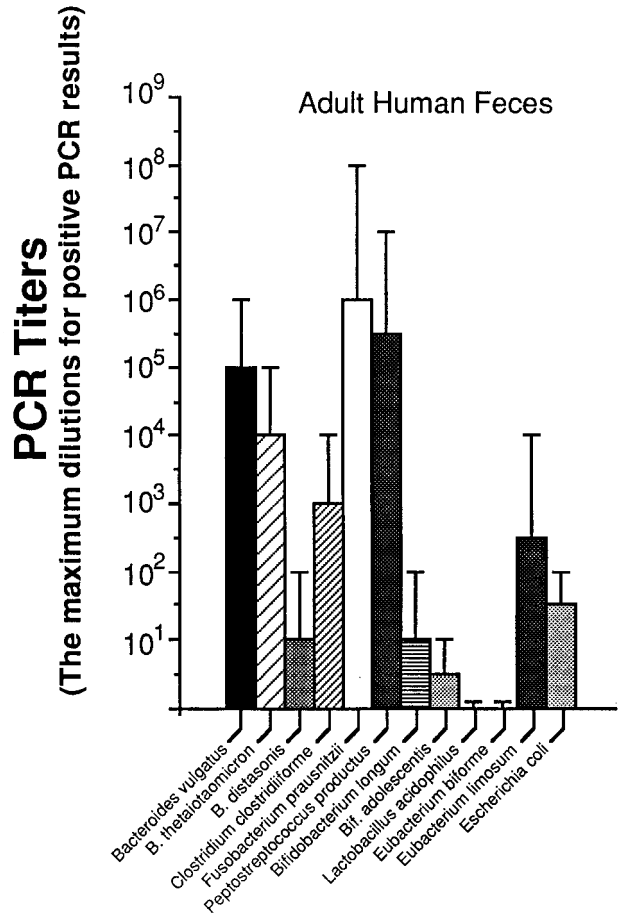

FIG. 3. PCR titers (the maximum dilutions for positive PCR results) for 12 intestinal bacterial species in human fecal samples are shown. The error bars show the ranges above the average values.

DISCUSSION

To complete comprehensive studies on the effect of dietary changes, food additives, and antimicrobial residues in food on the composition of intestinal microflora, rapid, sensitive, and specific methods for the detection of intestinal microbes are needed. Therefore, 12 PCR-based probes were developed for the quantitative detection of the predominant intestinal microflora in human and animal samples. The PCR results are in agreement with the studies using the more laborious and time-consuming traditional methods for the detection of intestinal microbes in mixed populations. For example, a fecal sample (adult human fecal sample 3) had a total of 55 anaerobe colonies according to the fecal sample culture. Fifty-five percent of the colonies were identified to be *Bacteroides* spp., 26% were *Eubacterium* spp., and some of the colonies were identified to be *Fusobacterium* spp. and *Peptostreptococcus* spp., but 17 (31%) of the colonies did not grow in the subculture. The PCR titers from this fecal sample (Table 3) were as follows: for *Bacteroides vulgatus* and *Bacteroides thetaiotaomicron*, 10^{-6} and 10^{-5}, respectively; for *F. prausnitzii*, 10^{-6}; for *P. productus*, 10^{-6}; and for *Eubacterium limosum*, 10^{-4}. Thus, the PCR method correlates well with the traditional culture method. Many reports on the quantification of fecal floras have been published (9, 13, 15, 16). In these reports, *Bacteroides vulgatus* and *F. prausnitzii* were often ranked first and second for the percentage of isolates or for the estimated counts per gram of fecal dry matter. *Bacteroides thetaiotaomicron*, *P. productus*, and *Eubacterium* spp. were also listed as the 10 most predominant species in the fecal flora. The PCR detection methods confirm these observations. In addition, the PCR titer for *E. coli* in feces was 100 to 100,000 times lower than those for the above-mentioned species, which is in agreement with the earlier reports; i.e., *E. coli* is a minor part of the microbial population in the gastrointestinal tract (9, 15).

Some differences between the results of PCR and the culture method were observed. For example, *C. clostridiiforme* had a high PCR titer in our test for all human and animal fecal samples, but *C. clostridiiforme* is not often listed as a predominant species by culture methods (9, 15, 16). One reason for the difference is that the PCR method in this investigation detects bacteria in situ, whereas culture methods detect bacteria after enrichment. Some species are unculturable or more difficult to grow than other species, which makes enumerations by the culture method growth dependent. However, PCR could detect unculturable or dead bacterial cells in situ. Thus, in this regard PCR is a better method than the culture method. On the other hand, PCR methods for some species were not very sensitive. For example, the PCR sensitivity for *Bifidobacterium adolescentis* was 10,000 cells, which makes PCR for quantification of this species not very sensitive. Hopefully, PCR methods for these species will be further improved in future studies.

Kreader (13) suggested that *Bacteroides thetaiotaomicron* could be used as an indicator for water contamination to distinguish human from nonhuman contamination sources. Our data confirm that this species was at higher titers in all adult human fecal samples, but was at lower titers or was not detected in animal fecal samples. However, *F. prausnitzii*, *P. productus* and *C. clostridiiforme* were the three species with the highest PCR titers in all human and animal fecal samples tested; therefore, the PCR procedures for these three species

TABLE 3. Quantitative detection of 12 bacterial species in human and animal fecal samples by PCR

Sample[a]	PCR result with primer set[b]:											
	BV	BT	BD	CC	FPR	PSP	BIL	BIA	LAA	EBI	ELI	ECO
AH-1	−6	−5	−	−4	−4	−4	−1	−1	−	−	−2	−1
AH-2	−6	−4	−	−3	−5	−6	−	−	−	−	−	−2
AH-3	−6	−5	−2	−4	−6	−6	−2	−1	−	−	−4	−1
AH-4	−6	−5	−	−3	−5	−7	−	−1	−	−	−2	−2
AH-5	−4	−3	−1	−3	−6	−5	−	−	−	−	−2	−2
AH-6	−4	−3	−1	−3	−6	−5	−1	−	−	−	−1	−1
AH-7	−6	−4	−1	−4	−8	−5	−	−	−	−	−2	−
BH-1	−	−2	−	−3	−2	−6	−3	−	−	−	−1	−2
BH-2	−	−	−	−3	−7	−4	−4	−	−2	−	−1	−1
Mouse-1	−	−	−	−2	−3	−3	−1	−	−1	−	−1	−
Mouse-2	−	−2	−	−3	−3	−4	−1	−	−1	−	−1	−
Rat-1	−	−2	−	−3	−3	−3	−3	−	−1	−	−1	−
Rat-2	−	−1	−	−3	−4	−4	−1	−	−	−	−1	−
Cat-1	−2	−1	−	−2	−4	−3	−	−	−	−	−	−2
Cat-2	−3	−	−	−3	−4	−3	−1	−	−	−3	−1	−1
Dog	−2	−1	−	−3	−4	−3	−1	−1	−	−3	−1	−1
Monkey	−	−	−	−2	−4	−4	−	−	−1	−1	−1	−1
Rabbit	−1	−	−	−2	−3	−3	−	−	−	−	−	−

[a] Fecal samples were from adult humans (AH-1 to -7), baby humans (BH-1 and -2), BALB/c mice, Fischer rats, a rhesus monkey, and a New Zealand rabbit.
[b] Abbreviations for PCR primer sets: BV, *Bacteroides vulgatus*; BT, *Bacteroides thetaiotaomicron*; BD, *Bacteroides distasonis*; CC, *C. clostridiiforme*; FPR, *F. prausnitzii*; PSP, *P. productus*; BIL, *Bifidobacterium longum*; BIA, *Bifidobacterium adolescentis*; LAA, *L. acidophilus*; EBI, *Eubacterium biforme*; ELI, *Eubacterium limosum*; ECO, *E. coli*. PCR results: −, PCR negative at a 10^{-1} dilution; −1 to −8, the maximum dilutions (10^{-1} to 10^{-8}) that yielded positive PCR results.

might also be useful as replacements for the fecal coliform assays as indicators of fecal contamination in drinking water.

The most prevalent bacterial species in the feces of experimental animals (the rats, mice, and rhesus monkey) and house pets (the cats, dog, and rabbit) screened varied qualitatively and quantitatively with the species of mammal. This is not surprising since the physicochemical conditions are different between animal species and humans (10, 13).

A simplified procedure to remove the PCR-interfering materials from feces and the introduction of direct PCR on cells without lengthy DNA purification are reported in this article. Feces contain many compounds, such as bilirubin and bile salts, which can inhibit PCR analysis (13, 26). PCR detection of bacteria in feces usually requires DNA purification. For example, Kreader (13) used a procedure including proteinase K-hexadecyltrimethylammonium bromide (CTAB)-phenol extraction to isolate DNA. The isolated DNA still contained PCR inhibitors. It was recommended that a glass-milk method for further purification of the DNA should be used. The DNA isolation and purification procedures are time-consuming; thus, the author indicated that simpler sample preparation procedures must be developed before PCR can become a general monitoring or diagnostic tool (13).

The procedure reported in this work is very simple and efficient. The centrifugation and washing steps are enough to remove the PCR inhibitors from feces. In contrast, without the centrifugation and washing steps, all these PCR assays gave negative results for fecal samples (data not shown). However, PCR results obtained by the procedure reported in this article showed no interfering substances.

Boiling the bacterial cells in 1% Triton X-100 and then immediately cooling them in ice-water was efficient for releasing and denaturing the DNA template. The resulting materials were directly subjected to the PCR assay without DNA isolation and purification steps. This procedure has worked well for a wide phylogenetic spectrum of bacteria and viruses (18–25).

In conclusion, PCR procedures for quantitative detection of 12 bacterial species predominant in human and animal feces were developed. The PCR methods with simple fecal-sample preparation procedures could be helpful for studies on the role of intestinal bacteria in xenobiotic metabolism and in safety evaluation of various food additives.

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