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Oligonucleotide Probes That Detect Quantitatively Significant Groups of Butyrate-Producing Bacteria in Human Feces

Georgina L. Hold,[1]* Andreas Schwiertz,[2]† Rustam I. Aminov,[1] Michael Blaut,[2] and Harry J. Flint[1]

Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, United Kingdom[1], and Deutsches Institut für Ernaehrungsforschung, Abteilung Gastrointestinale Mikrobiologie, 14558 Bergholz-Rehbrücke, Germany[2]

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16S rRNA-targeted oligonucleotide probes were designed for butyrate-producing bacteria from human feces. Three new cluster-specific probes detected bacteria related to *Roseburia intestinalis*, *Faecalibacterium prausnitzii*, and *Eubacterium hallii* at mean populations of 2.3, 3.8, and 0.6%, respectively, in samples from 10 individuals. Additional species-level probes accounted for no more than 1%, with a mean of 7.7%, of the total human fecal microbiota identified as butyrate producers in this study. Bacteria related to *E. hallii* and the genera *Roseburia* and *Faecalibacterium* are therefore among the most abundant known butyrate-producing bacteria in human feces.

The microbiota of the gastrointestinal tract of humans has been studied extensively because of the role played by gut bacteria both in disease and in the maintenance of gut health (7, 17, 27). One important activity of the large intestinal microbiota is to break down substrates, such as resistant starch and plant cell wall polysaccharides. The main fermentation products are the short-chain fatty acids acetate, propionate, and butyrate. Of these, butyrate is known to play an important role in the metabolic welfare of colonocytes (19, 20) and is also implicated in providing protection against cancer and ulcerative colitis (3–5). Despite this prominent role, the taxonomy, population structure, and dynamics of predominant butyrate-producing bacteria in the human intestinal tract are poorly understood.

There is no simple way to selectively isolate butyrate-producing bacteria, and the majority of those recovered from nonselective isolation have proved to be highly oxygen sensitive (2). The purpose of the present study was, therefore, to design 16S rRNA-targeted oligonucleotide probes for butyrate-producing bacteria, including recent isolates from the human gut, many of which represent new species (8, 9, 21). The majority of these butyrate-producing isolates belong to the clusters XIVa and IV of clostridia (6) (Fig. 1 and 2), which account for a significant proportion of total bacterial diversity in the human large intestine on the basis of 16S rRNA sequence analyses (12, 25).

Four broad-specificity probes were designed to target the small subunit rRNA of bacteria related to *Eubacterium hallii*, the recently reclassified *Faecalibacterium prausnitzii* (formerly *Fusobacterium prausnitzii*) (9), *Coprococcus eutactus*, and *Roseburia intestinalis* clusters (Table 1 and Fig. 1 and 2). The latter probe is predicted to recognize *R. intestinalis*, *Eubacterium rectale*, and *Eubacterium ramulus* as well as the butyrate-producing *Eubacterium* isolates A2-194 and L1-83 and the *Roseburia* isolate A2-183 from the study of Barcenilla et al. (2). In addition, six more specific probes were designed to recognize *R. intestinalis* (8), *Anaerostipes caccae* (21), the *Eubacterium* isolates L1-83 and A2-194, *Coprococcus* isolate L2-50, and *E. rectale* isolate A1-86. The new probes were designed with the ARB (16) software package, checked against the Ribosomal Database Project (RDP) and EMBL databases, and named according to the nomenclature suggested by the Oligonucleotide Probe Database (OPD) (1). The probe sequences have also been deposited in the ProbeBase data bank (15). The specificity of the newly designed probes was tested by whole-cell in situ hybridization against a panel of 120 reference strains derived from the human and animal gastrointestinal tract as described by Schwiertz et al. (22) and also against the new target butyrate-producing strains. Hybridization temperatures (T_H) are given in Table 1. All newly designed probes hybridized only to the respective target organisms but not to any of the other organisms tested. The exception was the L1-83 probe, which showed weak cross-reactivity with *Eubacterium* sp. strain A2-194.

Fresh fecal samples from 10 healthy volunteers of both sexes, aged 28 to 56, who had consumed a Western diet and had not received any antibiotic treatment at least 3 months prior to the study, were collected and fixed as described elsewhere (24). Hybridization and enumeration were performed as described previously (22), with the lower limit of detection of 10^7 cells g^{-1} of dry feces. In addition to analysis with the newly designed probes described above, the fecal samples were analyzed with 11 *Eubacterium* species-specific probes described previously (22–24). Broad-specificity probes or probe mixes that targeted all eubacteria were applied (14), along with the *Ruminococcus-Eubacterium-Clostridium* cluster probe (Erec482) (10), the *Clostridium lituseburense* group probe (Clit135) (13), the *Clostridium histolyticum* group probe (Chis150) (10), and the *Eubacterium cylindroides* group probe (Ecyl387) (11).

Cell counts for the target organisms in the 10 subjects are summarized in Table 2. Each subject harbored at least three groups of butyrate producers, with a mean of 7.7% of the total fecal microbiota identified as butyrate producers in this study. The Fpra655 (*F. prausnitzii*) probe detected between 1.4 and 5.9% (mean, 3.8%) of the total fecal microbiota in all 10

* Corresponding author. Present address: Department of Medicine and Therapeutics, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, United Kingdom. Phone: 44 (0) 1224 555980. Fax: 44 (0) 1224 554761. E-mail: g.l.hold@abdn.ac.uk.

† Present address: SymbioHerborn Group, D-35745 Herborn, Germany.

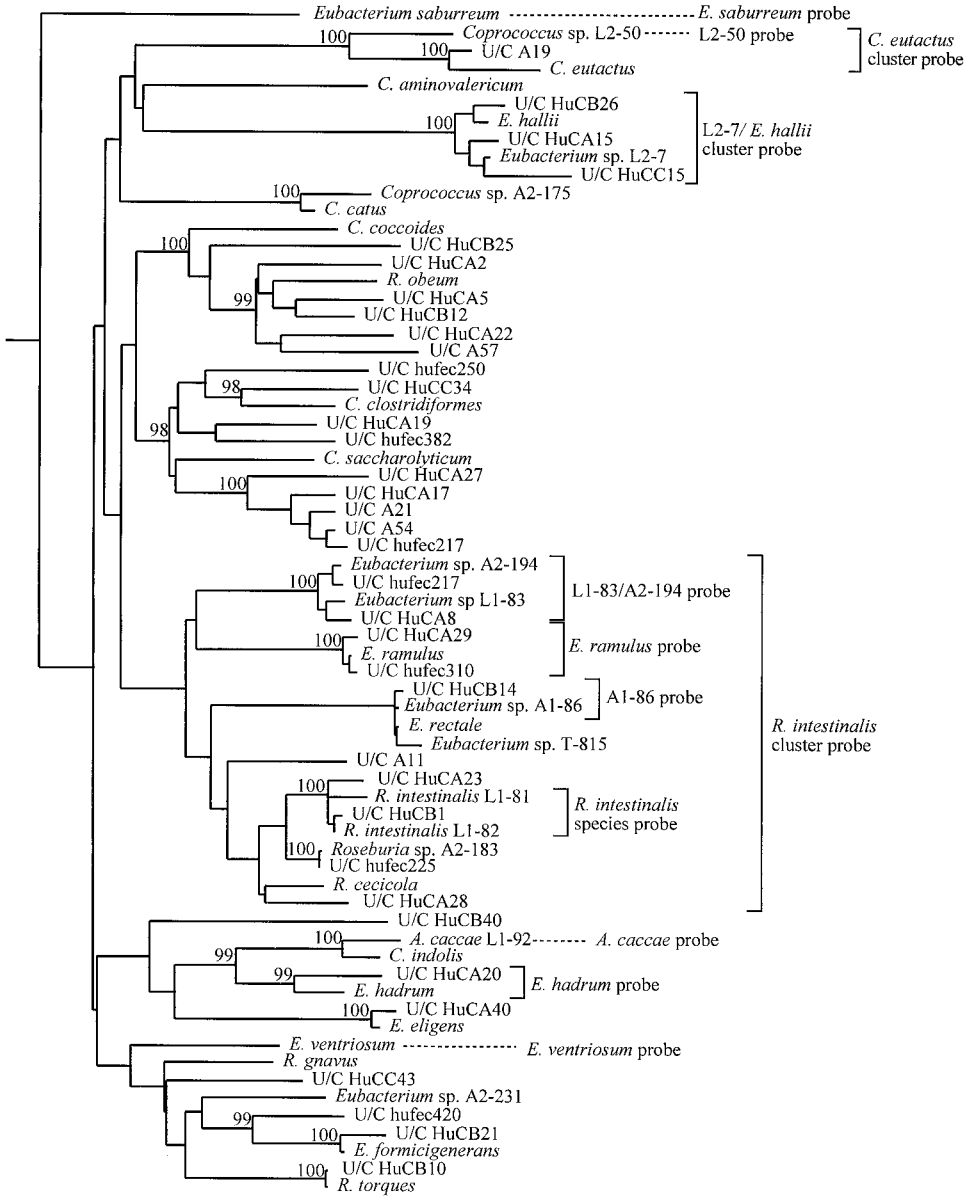

FIG. 1. Phylogenetic tree showing the coverage of the newly designed probes within *Clostridium* cluster XIVa. The tree was constructed by using neighbor-joining analysis of a distance matrix obtained from a multiple-sequence alignment. Bootstrap values (expressed as percentages of 1,000 replications) are shown at branch points; values of 97% or more were considered significant.

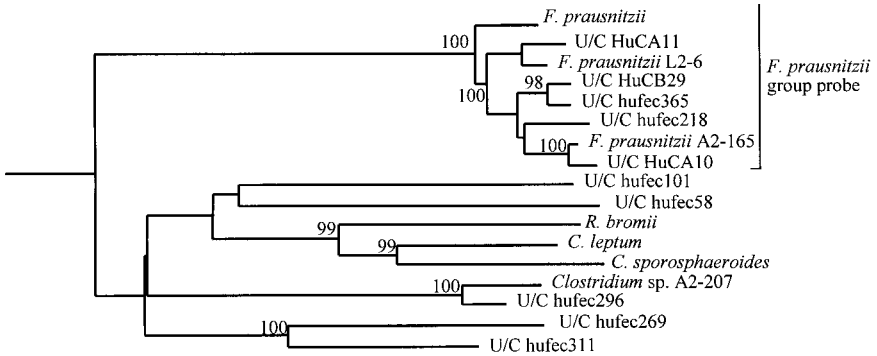

FIG. 2. Phylogenetic tree showing the coverage of the newly designed *F. prausnitzii* probe within *Clostridium* cluster IV. The tree was constructed by using neighbor-joining analysis of a distance matrix obtained from a multiple-sequence alignment. Bootstrap values (expressed as percentages of 1,000 replications) are shown at branch points; values of 97% or more were considered significant.

TABLE 1. Probes used during the study

Probe name	OPD name[a]	Target species with zero mismatches	Probe sequence 5′-3′	T_H (°C)	Reference or source
Species-specific probes					
A. caccae	S-S-Acac-0194-a-A-18	*A. caccae*	CTA TAC TGC CAG GGC TTT	46	This study
R. intestinalis	S-S-Rint-1102-a-A-18	*R. intestinalis*	GCT TAC CCG CTG GCT ACT	46	This study
Eubacterium sp. strain L1-83[b]	S-St-xxxx-0576-a-A-18	*Eubacterium* sp. strains L1-83 and A2-194	AGC CTT CCG CCT GCG CTC	58	This study
Coprococcus sp. strain L2-50[b]	S-St-xxxx-0060-a-A-18	*Coprococcus* sp. strain L2-50	CAC CGA TCT TCT CTC GTT	54	This study
E. rectale sp. strain A1-86[b]	S-S-Erec-0207-a-A-18	*E. rectale* strain A1-86	GGT GGT GTA CAA GAC CCG	52	This study
E. barkeri	S-S-Ebar-1237-a-A-18	*E. barkeri, E. aggregans*	CCT TTG TCC CAA CCC ATT	51	22
E. biforme	S-S-Ebif-0462-a-A-18	*E. biforme*	CAC TCA CTC ATC ATT CCC	51	22
E. cylindroides	S-St-Ecyl-0461-a-A-18	*E. cylindroides*	ACC CAC GGA TCA TTC CCT	51	22
E. cylindroides	S-St-Ecyl-0466-a-A-18	*E. cylindroides*	CCG TCA CCC ACA TAG CAT	51	22
E. hadrum	S-*-Ehad-0579-a-A-20	*E. hadrum*	GAC TTG CCA TAC CAC CTA CG	54	22
E. limosum	S-*-Elim-1433-a-A-18	*E. callanderi, E. limosum*	GCG GTT CTC TCA CAG GCT	51	22
E. moniliforme	S-S-Emon-0084-a-A-18	*E. moniliforme*	CCG CTA ATC CAT TTC CCG	51	23
E. multiforme	S-S-Emul-0183-a-A-18	*E. multiforme*	GTT CCT TCA TGC GAA GGT	51	23
E. ramulus	S-S-Eram-0997-a-A-18	*E. ramulus*	ACA TGT TCT GTC ACC GGG	46	24
E. saburreum	S-S-Esab-1467-a-A-18	*E. saburreum*	AGT TAT CCT CCC TGC CTT	48	23
E. ventriosum	S-S-Even-0066-a-A-18	*E. ventriosum*	TCT GTC CAA GGT GCT TCG	55	22
Group-and cluster-specific probes					
E. hallii L2-7/ *E. hallii*	S-*-Ehal-0578-a-A-18	*E. hallii* L2-7, *E. hallii*	TTG CAC TGC CAC CTA CGC	58	This study
E. cylindroides cluster	S-*-Ecyl-0387-a-A-18	*C. innocuum, E. biforme, E. cylindroides, E. dolichum, E. tortuosum, Streptococcus pleomorphus*	CGC GGC ATT GCT CGT TCA	46	11
Ruminococcus-Eubacterium-Clostridium cluster	S-*-Erec-0482-a-A-19	For details see Franks et al. (10)	GCT TCT TAG TCA RGT ACC G	50	10
C. eutactus	S-*-Ceut-0705-a-A-21	*C. eutactus, Coprococcus* sp. strain L2-50	GTC AGT AGC AGT CCA GTA AGT	54	This study
F. prausnitzii	S-*-Fpra-0655-a-A-18	*F. prausnitzii* A2-165 and L2-6	CGC CTA CCT CTG CAC TAC	58	This study
R. intestinalis subcluster	S-*-Rint-0623-a-A-18	*Eubacterium* sp. strains L1-83 and A2-194, *E. rectale* sp. strains A1-86, T1-815, and *Roseburig* sp. strain A2-183, and *R. cecicola, R. intestinalis, E. rectale, E. ramulus*	TTC CAA TGC AGT ACC GGG	54	This study
C. histolyticum	S-*-Chis150-a-A-23	For details see Franks et al. (10)	TTA TGC GGT ATT AAT CTY CCT TT	50	10
C. lituseburense	S-*-Clit135-a-A-19	For details see Franks et al. (10)	GTT ATC CGT GTG TAC AGG G	50	10

[a] Standardized probe name in accordance with the OPD (1).
[b] No valid systematic name was available.

subjects, which is in agreement with previous evidence, by using a different probe, indicating that this is one of the most abundant species in human feces (26). Also found in all 10 subjects was the *R. intestinalis* cluster (by using the Rint603 probe), which accounted for 0.9 to 5.0% (mean, 2.3%) of the total microbiota. Thus, the *R. intestinalis* and *F. prausnitzii* groups, which are likely to consist largely if not wholly of butyrate-producing strains, together accounted for not less than 3% and up to 10.9% (mean, 6.1%) of total eubacterial cells in the subjects studied. Organisms detected by the Ehal578 (*E. hallii*) probe were also widespread, being found in nine subjects and accounting for up to 2.4% (average of 0.6%) of the total microbiota. Recent work by Harmsen et al. (11) with a different probe showed that *E. hallii* and its close relatives can account for up to 3.6% of the total human fecal microbiota.

The species-specific probes for *R. intestinalis* and *Eubacterium hadrum* were positive for eight subjects. Bacteria related to *Eubacterium* sp. strains L1-83 and A2-194 and *E. ramulus* were each found in six subjects, while relatives of *E. rectale* sp. strain A1-86 were detected in four subjects. *Eubacterium biforme* and *Eubacterium ventriosum* were detected in only two subjects, and *Coprococcus* sp. strain L2-50 was detected in only one subject. In a PCR-based analysis the *Coprococcus* cluster was shown to account for up to 8% of total bacterial diversity in one human individual (25). Species probes for *Anaerostipes*

caccae, *Eubacterium barkeri*, *E. cylindroides*, *Eubacterium dolichum*, *Eubacterium saburreum*, *Eubacterium limosum*, *Eubacterium moniliforme*, and *Eubacterium multiforme* failed to detect cells above the limit of detection in samples from any of the 10 subjects. Negative results were also obtained with the cluster probes Chis150 and Clit135, but the Ecyl387 group probe detected significant numbers in two individuals.

The Erec482 probe used for the detection of the whole *Ruminococcus-Eubacterium-Clostridium* cluster (cluster XIVa) detected between 5.2 and 26.4% of total fecal bacteria in the 10 volunteers tested here. These numbers are essentially in agreement with previously published data (10, 22, 23). Nonoverlapping probes designed to recognize butyrate-producing species within the Erec482 cluster, namely Rint603, Ehal578, Ehad579, and Event66, together accounted for between 10.2 and 85% (mean, 43%) of the Erec482 signal. Thus, in some subjects (subjects 2, 4, and 7) almost all of the Erec482 representatives were closely related to known butyrate producers, while in others the remaining Erec482 diversity may correspond to species that do not produce butyrate (e.g., *Ruminococcus* sp.) but might also include groups of butyrate producers that have yet to be targeted.

We have now designed and validated probes that target most of the presently known butyrate-producing species or groups from the human gut within the *Clostridium* clusters IV and XIVa. The main conclusion is that butyrate producers accounted for, on average, 7.7% of the bacteria in the 10 subjects studied, with the most abundant groups by far being *R. intestinalis* and *F. prausnitzii*. Interestingly, the proportion of bacterial cells belonging to *Clostridium* cluster XIVa was lower in this set of volunteers than for those of previously published data sets (10, 18). This highlights interindividual differences possibly due to diet or geographic location. Also, the fact that the narrower strain or species-specific probes tested here did not detect bacteria in all fecal samples further emphasizes the diversity of the colonic microbiota at the strain level.

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TABLE 2. Quantification of the various bacterial components known to produce butyric acid within the human fecal microbiota[a]

Target	Probe[b]	No. of cells/g (dry wt) and percentage of the total microbiota in volunteer no.[b]									
		1	2	3	4	5	6	7	8	9	10
All eubacteria	Universal	11.7	11.8	11.6	11.8	12.2	11.7	12	11.7	11.5	11.9
Ruminococcus-Eubacterium-Clostridium cluster	Erec482	10.7 (5.2)	10.7 (8.06)	11 (26.4)	10.5 (5.2)	10.9 (5.3)	10.9 (15)	10.8 (6.3)	10.6 (8.4)	10.8 (19.7)	10.9 (8.8)
E. hallii cluster	Ehal578	8.8 (0.13)	10.1 (2)		8.3 (0.03)	9.1 (0.1)	9 (0.17)	10.4 (2.4)	8.8 (0.12)	9.2 (0.48)	9 (0.12)
R. intestinalis cluster	Rint603	9.6 (0.87)	10.4 (3.88)	10 (2.6)	10 (1.66)	10.6 (2.2)	10 (2.07)	10.4 (2.74)	9.8 (1.22)	10.2 (4.98)	10 (1.16)
R. intestinalis	Rint1102	8.4 (0.05)	8.4 (0.03)		9.5 (0.44)	9.5 (0.22)	9.4 (0.47)		8.5 (0.06)	8.8 (0.2)	9.2 (0.2)
Eubacterium sp. strains L1-83 and A2-194			8.4 (0.03)			10.1 (1.28)	9.1 (0.24)		9.4 (0.51)	8.8 (0.2)	9.4 (0.26)
E. rectale sp. strain A1-86	Erec207	8.8 (0.12)					8.5 (0.06)	8.5 (0.04)			9.5 (0.34)
E. ramulus	Eram997		8.5 (0.05)	8.1 (0.03)		8.9 (0.05)	8.9 (0.01)		8 (0.02)		8.6 (0.05)
Coprococcus sp. strain L2-50						8.8 (0.035)					
E. hadrum	Ehad579	9.1 (0.4)	9.1 (0.2)	8.5 (0.1)	9 (0.14)	9.3 (0.01)	9.5 (0.56)	9.2 (0.17)			9.2 (0.2)
E. ventriosum	Event66				10.1 (1.7)						10.2 (1.7)
E. cylindroides group	Ecyl387		10.2 (2.5)						9.9 (1.7)		
E. biforme	Ebif462		10.2 (2.5)						9.9 (1.7)		
F. prausnitzii cluster	Fpra655	10.1 (2.7)	10.5 (4.6)	10.2 (4.1)	10.4 (3.75)	10.8 (3.6)	10.5 (5.53)	10.1 (1.35)	10.5 (5.89)	10.2 (4.9)	10.2 (1.8)

[a] All probes were negative for *E. barkeri*, *E. multiforme*, *E. moniliformе*, *E. saburreum*, *E. cylindroides*, *E. dolichum*, *E. limosum*, *A. caccae*, *C. lituseburense*, and *C. histolyticum*.

[b] Counts of bacteria are expressed as numbers of organisms $\log_{10}$ per gram of feces (dry weight). Coefficient of variation due to assay error of the fluorescent in situ hybridization method was 0.18 (13). The percentage of the total microbiota was calculated by using counts from the universal eubacterial probe (1H).

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