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## Colonic Microbiota Signatures across Five Northern European Countries

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**The composition of the colonic microbiota of 91 northern Europeans was characterized by fluorescent in situ hybridization using 18 phylogenetic probes. On average 75% of the bacteria were identified, and large inter-individual variations were observed. *Clostridium coccoides* and *Clostridium leptum* were the dominant groups (28.0% and 25.2%), followed by the *Bacteroides* (8.5%). According to principal component analysis, no significant grouping with respect to geographic origin, age, or gender was observed.**

For the past 10 years, the progress made in molecular technologies has given rise to new ways to explore the human colonic microbiota. Investigations based on 16S rRNA sequences have revealed the presence of hundreds of molecular species, the majority uncultivated and/or not yet cultivated, unique to their host and with few species shared between two individuals (5, 10, 13). Fluorescent in situ hybridization (FISH) (1) of the 16S rRNA has shown that the species diversity comprised less than 20 dominant phylogenetic groups (2–4, 7). However, the molecular analysis has so far been restricted to limited cohorts of individuals, recruited within a single geographic region or country (3, 4, 7, 8, 14). In this study, we characterized the fecal microbiota of 91 individuals from five northern European countries to provide a large-scale molecular analysis of the normal colonic microbiota in healthy humans. Multivariate data analysis was performed in order to seek a possible link between the composition of the fecal microbiota and age, gender, or geographic origin parameters.

**Composition of the fecal microbiota assessed by FISH combined with flow cytometry.** Fecal samples were collected from 91 healthy humans aged between 7 and 52 years. These donors were from France ( $n = 21$ ), Denmark ( $n = 20$ ), Germany ( $n = 20$ ), The Netherlands ( $n = 20$ ), and the United Kingdom ( $n = 10$ ) and had a nonrestricted Western European diet. None had a history of digestive pathology nor had received antibiotic treatment within 6 months before the study. The fixation procedure was adapted from the study of Schwartz et al. (9). A set of 18 group- and species-specific probes defined previously (4) was applied to describe the fecal microbiota composition. Fluorescent in situ hybridization combined with flow cytometry was performed as described previously (4, 7, 8). The proportions of cells that hybridized with the phylogenetic probes

relative to the total bacteria are presented in Table 1. For the five European countries, the highest percentages were detected with the *Clostridium coccoides*-*Eubacterium rectale* probe (Erec 482) and the *Clostridium leptum* probe (Clep 866), representing 28.0% and 25.2% of total bacteria, respectively. These groups codominated in France, the United Kingdom, and Germany, compared to Denmark and The Netherlands, where the *Clostridium coccoides*-*Eubacterium rectale* group predominated. Within the *Clostridium leptum* subgroup the *Faecalibacterium prausnitzii* cluster (Ffrau 645) was the most abundant in all five countries, followed by the *Ruminococcus bromii* and related species (Rbro 730). The *Bacteroides* group (Bac 303) came third and accounted for 8.5% of bacterial cells. Members of the *Atopobium* (Ato 291) and the *Bifidobacterium* (Bif 164) groups represented 3.1% and 4.4% of the cells, respectively. The composition of the fecal microbiota obtained in this study correlated well with the composition observed in previous studies using FISH in combination with either microscopy and image analysis or flow cytometry (2–4, 7, 8, 11, 14). When the relative proportions of bacterial cells obtained with the panel of 12 nonoverlapping phylogenetic probes were added, a mean of 75.7% (ranging from 66.4% in France to 82.3% in The Netherlands) was calculated. Consequently, more than 24% of the human fecal microbiota still remained unidentified. Compared to the study of Rigottier-Gois et al. (7), the number of phylogenetic probes in the set was increased from 6 to 18, and the proportion of untargeted bacteria was thereby reduced from 49 to 24%. Nevertheless, efforts to characterize this phylogenetic gap must be continued. One approach will be to use the potential of flow cytometric cell sorting to select bacterial cells of interest (12), combined with molecular inventory to characterize the molecular species and thereby to design new probes.

**PCA of bacterial composition with biometric and geographic data.** All proportions of a given group or species as a function of geographic origin, age and gender were organized and subjected to principal component analysis (PCA) using the Un-

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TABLE 1. Proportions of the *C. coccooides*-*E. rectale* (Erec 482) group, the *C. leptum* (Clep 866, Cvir 1414, Edes 635) subgroup, and the *Bacteroides* (Bac 303), *Atopobium* (Ato 291), *Bifidobacterium* (Bif 164), *Lactobacillus-Enterococcus* (Lab 158), *Eubacterium cylindroides* (Ecy1 387), *Veillonella* (Veil 223), *Streptococcus* (Strc 493), and *Enterobacteriaceae* (Enter 1432) groups in healthy adults assessed with a combination of FISH and flow cytometry<sup>c</sup>

Probe	% of bacteria that hybridized with probe					
	France (n = 21)	Denmark (n = 20)	The Netherlands (n = 20)	United Kingdom (n = 10)	Germany (n = 20)	Europe (n = 91)
Erec 482	22.9 ± 9.9	33.0 ± 11.6	30.9 ± 10.1	25.4 ± 11.4	26.9 ± 11.3	28.0 ± 11.3
Clep 866 <sup>a</sup>	21.7 ± 7.7	26.3 ± 5.2	26.3 ± 7.3	25.1 ± 8.3	26.8 ± 8.5	25.2 ± 7.6
Rbro 730 <sup>a</sup>	2.7 ± 3.1	6.6 ± 5.7	4.8 ± 4.6	8.9 ± 7.1	10.2 ± 8.0	6.3 ± 6.4
Rfla 729 <sup>a</sup>	0.4 ± 0.6	1.3 ± 0.9	1.0 ± 1.1	0.5 ± 0.7	0.8 ± 0.9	0.8 ± 0.9
Rcal 733 <sup>a</sup>	0.3 ± 0.6	0.5 ± 0.6	0.5 ± 0.5	0.2 ± 0.5	0.1 ± 0.1	0.3 ± 0.5
Fprau 645	13.9 ± 9.0	16.2 ± 5.1	17.6 ± 6.6	13.1 ± 7.4	14.9 ± 7.1	15.4 ± 7.2
Cvir 1414 <sup>a</sup>	0.7 ± 0.5	0.8 ± 0.6	2.6 ± 1.9	2.2 ± 1.3	0.5 ± 0.8	1.3 ± 1.4
Edes 635	0.1 ± 0.1	0.2 ± 0.2	0.4 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2
Bac 303	7.2 ± 6.2	8.2 ± 7.7	9.5 ± 5.6	9.6 ± 7.9	8.7 ± 8.4	8.5 ± 7.1
Bfra 998	1.4 ± 1.3	0.8 ± 0.5	4.2 ± 3.3	2.1 ± 1.7	1.2 ± 1.4	1.9 ± 2.3
Bvulg 1017	1.7 ± 2.5	1.7 ± 2.4	1.1 ± 1.7	0.9 ± 1.8	4.0 ± 5.3	2.0 ± 3.3
Ato 291	4.8 ± 2.2	2.8 ± 1.8	2.6 ± 2.6	4.0 ± 4.4	1.7 ± 2.3	3.1 ± 2.8
Bif 164	4.4 ± 4.4	4.2 ± 4.4	3.6 ± 2.6	3.9 ± 1.9	5.8 ± 5.9	4.4 ± 4.3
Lab 158	2.0 ± 1.3	1.9 ± 1.4	1.4 ± 0.9	2.7 ± 2.2	1.4 ± 1.4	1.8 ± 1.4
Ecy1 387	1.1 ± 2.2	1.2 ± 1.7	1.7 ± 2.3	1.4 ± 2.0	0.4 ± 0.6	1.1 ± 1.9
Veil 223	0.9 ± 0.7	0.9 ± 0.9	2.5 ± 1.3	1.6 ± 1.9	0.9 ± 0.9	1.3 ± 1.3
Strc 493 <sup>a</sup>	0.4 ± 0.6	0.5 ± 0.4	0.8 ± 0.8	1.4 ± 1.6	0.6 ± 0.4	0.6 ± 0.8
Enter 1432	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.1 ± 0.1
Additivity <sup>b</sup>	66.4 ± 18.2	80.0 ± 16.5	82.3 ± 17.8	77.6 ± 20.5	73.7 ± 17.5	75.7 ± 18.6

<sup>a</sup> The indicated probes were combined with competitor oligonucleotides as described by Lay et al. (4).

<sup>b</sup> The species-specific probes Rbro 730, Rfla 729, Rcal 733, Fprau 645, Bfra 998, and Bvulg 1017 were excluded from the summation, since they detect groups of bacteria already covered by the probes Clep 866 and Bac 303.

<sup>c</sup> Members of the *C. leptum* subgroup are represented by *R. bromii* and related species (Rbro 730), *Ruminococcus flavefaciens* and related species (Rfla 729), *Ruminococcus callidus* (Rcal 733), and the *F. prausnitzii* cluster (Fprau 645), which are taken into account with the group probe Clep 866. The probes Cvir 1414 and Edes 635 target other members of the *C. leptum* subgroup (the *Clostridium viride* cluster and the *Eubacterium desmolans* species). Members of the *Bacteroides* group are taken into account with the group probe Bac 303 and are represented in part by the *Bacteroides fragilis* species (Bfra 998) and the *Bacteroides vulgatus* species (Bvulg 1017).

scrambler program version 7.6 (Cano AS, Trondheim, Norway). Altogether 91 individual fecal samples were analyzed in duplicate, giving a total of 182 data points in the PCA model.

The generated output of the PCA revealed that not very much of the variation could be described by individual principal components. These values suggested that no single or smaller

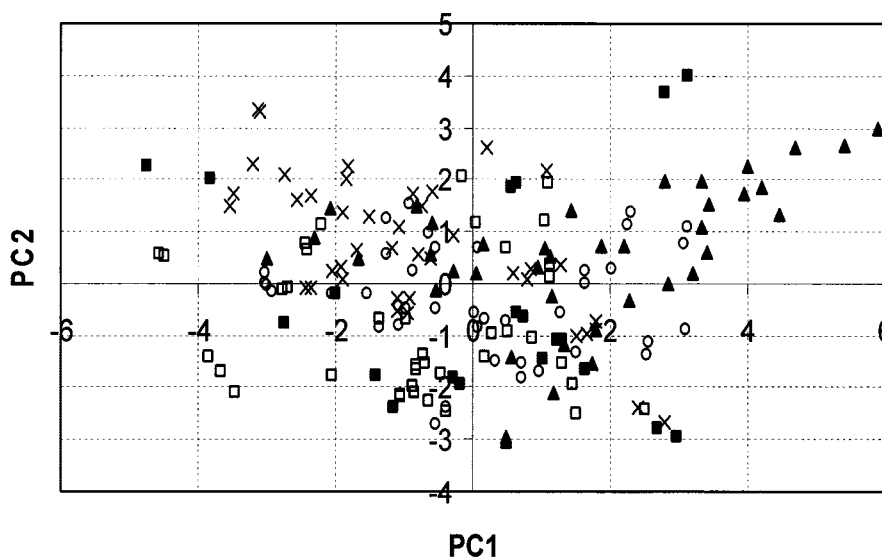


FIG. 1. Score plot of the PCA analysis with focus on groupings of samples with respect to geographic origin using principal components PC1 and PC2. The score plot shows the clustering of different samples, and samples that lie close to each other are similar with respect to the measured variables. Conversely, samples that lie diametrically opposite to each other will be very different from each other. Crosses, French; open squares, German; open circles, Danish; filled squares, English; filled triangles, Dutch.

groups of variables could assist in simplifying the interpretation of the data set. Consequently, results were evaluated for all six principal components, PC1 to PC6. The principal component analysis revealed large interindividual variations, showing that each individual harbors a specific colonic microbiota or signature in terms of bacterial composition. In our study, samples from one Danish family with two children were analyzed, and results showed that all members of the family are different. According to the PCA, no significant grouping of samples with respect to age or gender was observed, no matter whether the analysis was done for the entire cohort of samples or in a country-specific manner. No grouping of samples with respect to geographic origin was observed when the five countries were considered. However, as shown in Fig. 1 there is a tendency for the Dutch samples to accumulate in the right sector, while the French samples pull more in the left direction of the coordinate system, suggesting a weak difference between the French and Dutch samples. On the basis of the distribution of major dominant groups, the colonic microbiota of healthy humans thus appears comparable throughout the northern European countries investigated.

Nutrigenomics and nutrigenetics are two emerging fields of nutrition research, which seek to study the effects of nutrition on health and disease by examining the influence of dietary signatures (6). Microbial ecology of the gastrointestinal tract plays a key role in nutrition research into the relation between colonic microbiota signatures and age, diet, dietary allergies, or diseases. Molecular approaches based on 16S rRNA sequences provide tools for understanding this complex microbiota in relation to diet and human health. In this study, we showed that the colonic microbiota of healthy humans is comparable throughout the north of Europe. Targeting other ethnic populations will enable the composition of the human colonic microbiota around the world to be established. Performing such a study on infants and the elderly will facilitate the assessment of how the human digestive microbiota evolves with age. This baseline study on healthy humans will also contribute to eventually identifying changes in the composition of the intestinal microbiota in patients suffering from inflammatory bowel diseases.

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