Temperature gradient gel electrophoresis of fecal 16S rRNA reveals active Escherichia coli in the microbiota of patients with ulcerative colitis

Harry Sokol, Patricia Lepage, P. Seksik, Joël Doré, Philippe Marteau

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2006, p. 3172–3177 Vol. 44, No. 9
0095-1137/06/$08.00+0 doi:10.1128/JCM.02600-05

Temperature Gradient Gel Electrophoresis of Fecal 16S rRNA Reveals Active *Escherichia coli* in the Microbiota of Patients with Ulcerative Colitis

H. Sokol,[1] P. Lepage,[1] P. Seksik,[2]* J. Doré,[1] and P. Marteau[3]

INRA, UEPSD, CR de Jouy-en-Josas, 78352 Jouy-en-Josas, France[1]; Université Paris-VI Faculté de médecine, and AP-HP, Département d'Hépato-Gastroentérologie, Hôpital Saint-Antoine, Paris, France[2]; and Université Paris-Descartes, Faculté de médecine, and AP-HP, Département d'Hépato-Gastroentérologie, Hôpital Lariboisiere, Paris, France[3]

Received 14 December 2005/Returned for modification 31 January 2006/Accepted 16 July 2006

Previous studies of the endogenous microbiota in patients with ulcerative colitis (UC) have not taken bacterial activity into account, yet bacteria with high transcriptional activity might have a more important pathophysiological role than inactive bacteria. We therefore analyzed the biodiversity of active bacteria in the fecal microbiota of UC patients, in comparison with that of healthy subjects. Feces were collected from nine patients with active UC and from nine healthy controls. Total DNA and RNA were extracted, and 16S ribosomal DNA and RNA were amplified by PCR and reverse transcription-PCR, respectively. Amplification products were compared by means of temporal temperature gradient gel electrophoresis (TTGE). Bands of interest were excised, sequenced, and identified by comparison with the GenBank database (NCBI). The dominant-species diversity based on RNA-derived TTGE profiles was significantly lower for UC patients than for healthy controls ($P = 0.01$). The mean similarity index between the "present" and "active" microbiota was 74% ± 18% for UC patients. Comparison of the individual "active" microbiota identified a band that was present for eight UC patients and only two controls (89% versus 22%; $P = 0.008$). The band was sequenced for 6 patients and always corresponded to *Escherichia coli*. The biodiversity of active bacteria in the dominant fecal microbiota of patients with UC is lower than that of healthy subjects. *E. coli* is more represented in the active microbiota of UC patients. The possible pathophysiological role of this difference remains to be determined.

The cause of inflammatory bowel disease (IBD) is unknown, but the intestinal microbiota is considered to be the main, or at least a major, trigger of inflammation for both patients and animal models (21). Both the immune response to endogenous bacteria and the composition of the gut microbiota are thought to be abnormal in this setting (27), but the precise culprit, microbial species and/or microbial products, remains to be identified. The gut mucosal and fecal microbiota seem to differ both quantitatively and qualitatively between patients with ulcerative colitis (UC) and healthy controls (C). In particular, bacteria appear to be more abundant in the UC colonic mucosa (26). *Escherichia coli* strains with unusual adhesive or pathogenic properties have also been detected in some patients (28).

Temporal temperature gradient gel electrophoresis (TTGE), based on 16S rRNA gene electrophoresis, is more efficient than culture for identifying bacterial groups or species within the intestinal microbiota, since more than half of all colonic bacteria cannot be cultured (5, 24). However, while traditional methods detect the presence of bacteria in the fecal and mucosal ecosystems, they provide no information on their activity. Bacterial metabolic activity depends notably on genetic factors, the local ecology, and quorum sensing. Bacteria with high metabolic activity may play a more important role in disease onset and progression, since they may secrete or express more proinflammatory molecules, but this possibility has not yet been studied in relation to IBD. The rRNA content of bacterial cells closely reflects their transcriptional activity, and TTGE of rRNA has thus been used to identify active fecal bacteria (31).

The aim of this study was to analyze the biodiversity of active bacteria in the dominant fecal microbiota of UC patients in comparison with that of healthy subjects and to identify active bacterial species (based on their rRNA content) that may be more specifically associated with UC.

MATERIALS AND METHODS

Patients. Nine patients with active UC (five men and four women; mean age, 39 years [25 to 69]) and nine healthy controls (six men and three women; mean age, 43 years [23 to 69]) were studied with their informed consent. None of the patients had particular diets or eating habits. None of them had received antibiotics, sulfasalazine, or colon cleansing within the previous 3 months. Four subjects with UC had pancolitis, and the other five had left-sided colitis; two were receiving corticosteroids, five mesalazine, and one azathioprine.

Fecal sampling. Fecal samples were divided into aliquots in sterile Starstedt 2.2-ml screw-cap tubes and placed in liquid nitrogen within 1 h after their emission. They were then stored at −80°C until analysis.

TTGE. (i) Nucleic acid isolation and amplification. Total DNA was extracted from fecal samples as previously described (22, 25). RNA was extracted as described by Doré et al. (5). Nucleic acid concentration and integrity were determined visually by electrophoresis on a 1% agarose gel containing ethidium bromide. The PCR procedures described below were designed to amplify the V6 to V8 region of rRNA genes and also to check the RNA solutions for residual DNA. The primers GCclamp-U968 (5′ GCclamp-GAA CGC GAA GAA CCT TAC) and L1401 (5′ GCG TGT GTA CAA GAC CC) were used to amplify the V6 to V8 regions of bacterial 16S rRNA genes or rRNA, as previously described (14, 22). Reverse transcriptase-PCR (RT-PCR) was performed with the Geneamp Thermostable r*Tth* reverse transcriptase RNA PCR kit (Applied Biosystems, Foster City, Calif.) as described by Zoetendal et al. (31). Reverse transcriptase

* Corresponding author. Mailing address: Service de Gastroenterologie, Hopital St Antoine, 184 rue du Fg St Antoine, 75012 Paris, France. Phone: 01 49 28 31 64. Fax: 01 49 28 31 88. E-mail: philippe.seksik@sat.aphp.fr.

reaction mixtures (50 μl) contained 10 mM Tris-HCl (pH 8.3), 90 mM KCl, 1 mM $MnCl_2$, 200 μM (each) deoxynucleoside triphosphates, 5 U of r*Tth* DNA polymerase, 7.5 pmol of primer L1401, and 1 μl of 10- to 100-fold-diluted RNA (approximately 2 ng). The mixtures were incubated at 70°C for 15 min, and then 80 μl of PCR additive was added. The additive consisted of 4% glycerol, 8 mM Tris-HCl (pH 8.3), 80 mM KCl, 0.04% Tween 20, 0.6 mM EGTA, 3.75 mM $MgCl_2$, 50 mM (each) deoxynucleoside triphosphates, and 7.5 pmol of primer U968-GC. The samples were amplified in a PCT 100 thermocycler (MJ Research, Inc.) using the following program: 94°C for 1 min; 30 cycles of 94°C for 30 s, 56°C for 30 s, 68°C for 1 min, and finally 68°C for 7 min. PCR and RT-PCR products were analyzed by electrophoresis on a 1% agarose gel containing ethidium bromide in order to determine their sizes (~500 bp) and approximate concentrations.

(ii) TTGE analysis of PCR amplicons. We used the DCode universal mutation detection system (Bio-Rad, Paris, France) for sequence-specific separation of PCR products. Electrophoresis was performed as previously described (14, 22) at 64 mA for 16 h at an initial temperature of 66°C and a ramp rate of 0.2°C/h. To improve resolution, the voltage was set at 20 V for 15 min at the beginning of each run. Each well was loaded with 100 to 200 ng of amplified DNA plus an equal volume of 2× gel loading dye (0.05% bromophenol blue, 0.05% xylene cyanol, and 70% glycerol). We normalized the loadings to comparable quantities in order to analyze patterns with comparable intensities. Gels were stained in the dark by immersion for 30 min in a solution of SYBR Green I nucleic acid gel stain (Roche Diagnostics, GmbH, Mannheim, Germany) and were read using a Storm device (Molecular Dynamics).

Band analysis. The biodiversity of each sample was assessed from the number of bands in TTGE profiles. Bands representing DNA fragments of interest (500 bp) were removed from the gel with a 20-μl micropipette tip under UV illumination and were transferred to a 1.5-ml tube containing 200 μl of autoclaved water. After 4 min of centrifugation at 8,000 × *g*, the acrylamide pellet was resuspended in 100 μl of water and smashed before overnight diffusion. After 4 min of centrifugation at 8,000 × *g*, the supernatant containing DNA fragments was used for PCR reamplification with the same primers as above. The DNA integrity of amplicons was checked on a 1% agarose gel. To verify the correspondence between the DNA of interest on the first electrophoresis gel and the amplified DNA, we performed TTGE a second time, comparing the two samples. When the two bands comigrated, DNA fragments of interest were sent for sequencing (Genome Express, Meylan, France). The sequences were then compared to the GenBank database by using the BLAST program (Blastn, NCBI). When similarity indices between our sequences and previously described sequences exceeded 98%, we considered the sequences to correspond to the same species as the GenBank reference.

Calculations and comparisons. TTGE profiles were analyzed with Gel Compar software, version 2.0 (Applied Maths, Kortrigk, Belgium), as previously described (13). Similarity indexes (Pearson correlation method) were calculated for each pair of profiles (14, 22). Mean similarity indexes were first calculated for each patient and then for the overall study group. The results were compared by using Student's *t* test when the distribution was normal and otherwise with Wilcoxon's test. TTGE patterns were analyzed with GelCompar II software, which yields a spatial representation (dendrogram) based on the matrix of Pearson correlation coefficients, and by applying the unweighted pair group method using arithmetic averages (14, 22). The presence of the "active" *E. coli* band was analyzed by Fisher's exact test.

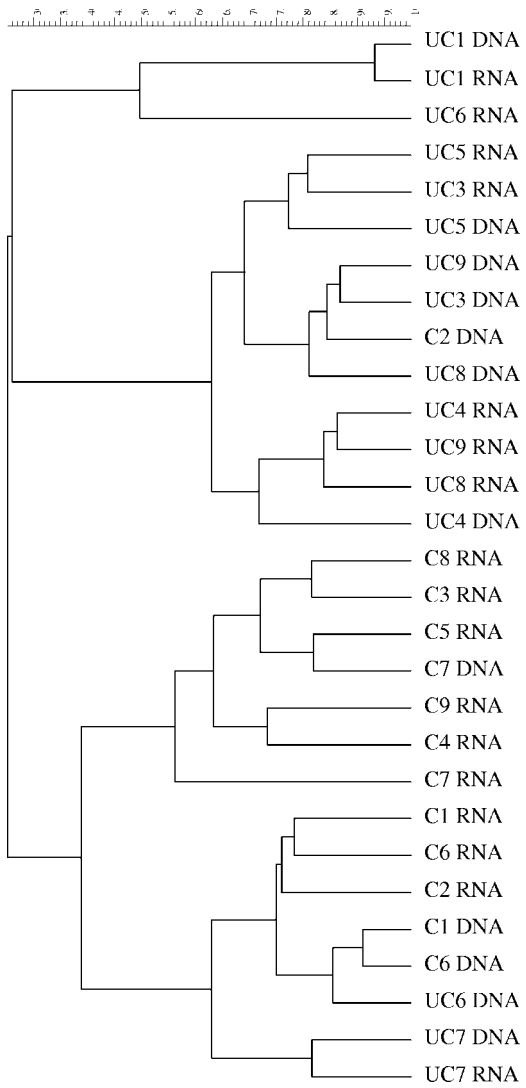

FIG. 1. Dendrogram representation of the TTGE profiles of 16S rRNa gene and rRNA amplicons (obtained using primers for the V6 to V8 regions) from fecal samples of nine UC patients and nine healthy controls. The dendrogram represents a statistically optimal representation of the similarities between TTGE profiles based on the matrix of Pearson correlation coefficients and by applying the unweighted pair group method using arithmetic averages. RNA- and DNA-derived TTGE profiles from a given patient did not cluster together, except for two patients with UC (UC1 and UC7). Samples tended to cluster on the basis of their clinical origin (UC versus control).

RESULTS

PCR, RT-PCR amplicons, and biodiversity. Among the 18 samples studied, total RNA was extracted from 18 (9 UC patients and 9 C), and total DNA was extracted from 12 (8 UC patients and 4 C). Hence, PCR amplification of the V6 to V8 regions of 16S rRNA genes was successful for 12/18 fecal samples, and RT-PCR amplicons of the same region were obtained for all 18 samples. The mean number of bands in DNA-derived TTGE profiles was 15.3 ± 3.2 and 18.3 ± 5.0 for UC and C, respectively ($P = 0.23$). The mean number of bands in RNA-derived TTGE profiles was 9.1 ± 2.8 bands and 14.7 ± 5.1 for UC and C, respectively ($P = 0.01$). The number of bands was significantly higher in DNA-derived profiles than in RNA-derived profiles for UC patients ($P = 0.001$) but not for controls ($P = 0.26$).

Overall dendrogram analysis. All TTGE profiles were compared, and the results were plotted as a single dendrogram. The branching distances between two samples shows their degree of relatedness in terms of the dominant species content. DNA- and RNA-derived TTGE profiles were thus compared for each subject on the same gel. Individual subjects' RNA- and DNA-derived TTGE profiles did not cluster together, except in two patients with UC (Fig. 1). Irrespective of the initial matrix (RNA or DNA), samples tended to cluster on the basis of their clinical affiliation (UC versus C). Except for one

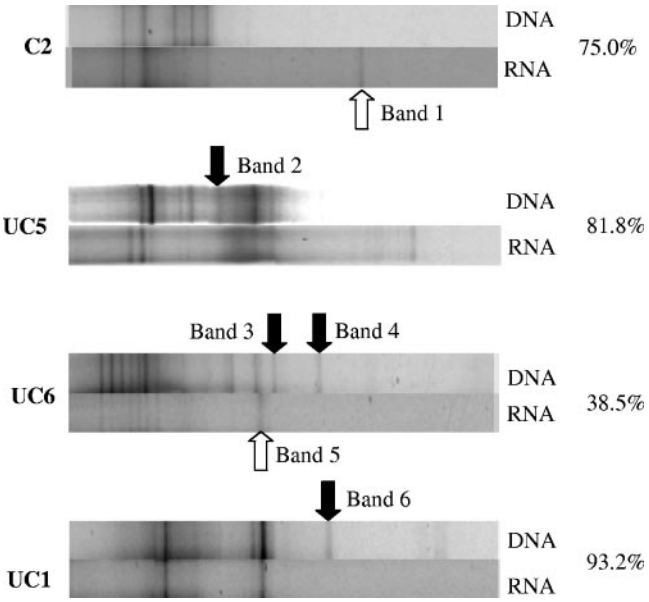

FIG. 2. TTGE of 16S rRNA gene and rRNA amplicons (obtained using primers for the V6 to V8 regions) from fecal samples from three UC patients and one control. Right side: similarity indexes (%) of paired samples. Black arrow: band present in the DNA-derived but not the RNA-derived TTGE profile. White arrow: band present in the RNA-derived but not the DNA-derived TTGE profile.

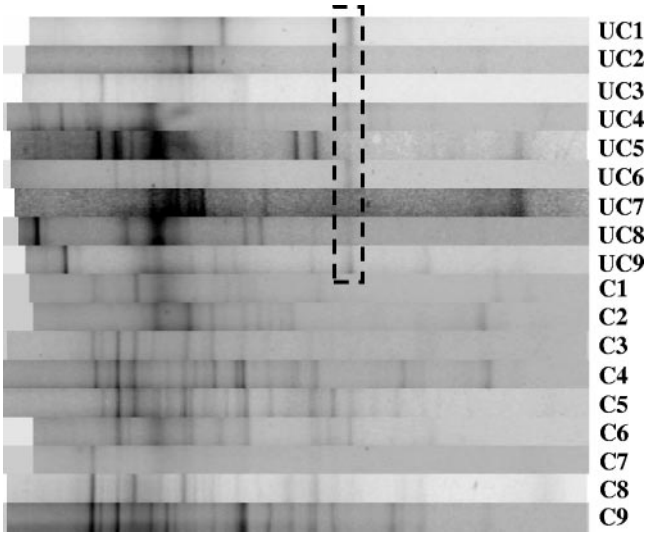

FIG. 3. TTGE of 16S rRNA amplicons (obtained using primers for the V6 to V8 regions) from fecal samples from nine UC patients and nine controls. One band was present for eight UC patients (framed) and only two controls.

DNA-derived amplicon, samples from the healthy controls formed a single cluster. Similarly, except for one patient and one DNA-derived amplicon from another patient, samples from patients with UC also formed a single cluster. This suggested that the UC and control groups each had specific bacterial signatures.

Intraindividual analysis. The average similarity index between DNA and RNA profiles was 74% ± 18% in UC and 75% ± 9% in C ($P = 0.9$). As shown in Fig. 2, some bands were more prominent in RNA-derived than in DNA-derived TTGE patterns, while some faint bands in RNA-derived patterns were prominent in the corresponding DNA-derived patterns.

Six bands were exclusively found in either the RNA or the DNA profiles of four subjects (3 UC and 1 C). These bands were sequenced and compared with the GenBank database (NCBI). Their characteristics are shown in Table 1.

TABLE 1. Characteristics of six bands excised and sequenced after fecal intraindividual comparisons (RNA- versus DNA-derived TTGE profiles)[a]

Subject	TTGE profile of origin	Best match in GenBank database	% Identity (BlastN)
UC2	RNA	*Neisseria flava*	98
UC5	DNA	Butyrate-producing bacterium T2-145	99
UC6	DNA	Butyrate-producing bacterium A2-175	99
UC6	DNA	Butyrate-producing bacterium SM6/1	99
UC6	RNA	*Escherichia coli*	99
UC1	DNA	*Acidaminococcus* sp.	100

[a] All six bands were specific for either the RNA- or DNA-derived TTGE pattern.

Interindividual analysis. Interindividual mean similarity indexes were compared among subjects for whom DNA and RNA TTGE profiles were available. The mean similarity index between TTGE DNA profiles was 58.2% ± 19% in UC and 61.3% ± 21% in C, whereas the mean similarity index between TTGE RNA profiles was 48.3% ± 15% in UC and 64.0% ± 13% in C. These differences between the patients and controls were not significant. The overall interindividual comparison of RNA profiles (Fig. 3) revealed a band that was present in 8/9 UC patients and in only 2/9 controls (prevalence, 89% versus 22%; $P = 0.008$). This band was removed from six of the eight patients' patterns, and the amplicons were sequenced and compared to the GenBank database (NCBI). Sequences of all the amplicons corresponded to the species *Escherichia coli* (similarity index always >98% with GenBank *E. coli* sequences) or related enterobacteria indistinguishable by 16S rRNA gene sequence (Table 2).

DISCUSSION

Alterations of the intestinal microflora are thought to be an important factor in IBD. The gut mucosa, lumen, and feces represent different ecosystems and normally harbor different microbiota (15). Detection of a given bacterium in an ecosystem by means of culture or 16S rRNA gene analysis does not necessarily mean that the bacterium has high metabolic activity. TTGE of 16S rRNA has previously been used to detect such active bacteria (31), but dysbiosis affecting active bacteria has never previously been examined with UC patients. However, a higher transcriptional activity corresponds to a higher cell replication rate and greater protein production. Our hypothesis is that the intensity of the proinflammatory signal from the microbiota could not only be related to the microbial biomass but also to bacterial activity. Recent findings (28)

TABLE 2. Characteristics of six bands excised after interindividual comparisons of fecal RNA-derived TTGE profiles[a]

Band	Subject	TTGE profile	Best match	% Identity (BlastN)
1	UC1	RNA	*Escherichia coli*, *Escherichia albertii*, *Escherichia fergusonii*, *Shigella boydii*, *Shigella flexneri*, *Photorhabdus luminescens*	100
2	UC2	RNA	*Escherichia coli*, *Shigella boydii*, *Salmonella* serovar Typhi, *Shigella sonnei*	99
3	UC4	RNA	*Escherichia coli*, *Escherichia albertii*, *Escherichia fergusonii*, *Shigella boydii*, *Photorhabdus luminescens*	98
4, 5	UC6, UC8	RNA	*Escherichia coli*, *Shigella boydii*, *Salmonella* serovar Typhi, *Shigella sonnei*	100
6	UC9	RNA	*Escherichia coli*, *Shigella boydii*, *Salmonella* serovar Typhi, *Shigella dysenteriae*	100

[a] All six bands were observed at the same level in the TTGE profile of each of the six subjects.

indicated that all dominant bacteria present in the intestinal microbiota did not have the same level of transcriptional activity; it hence seemed relevant to extend our investigation of bacteria associated with UC to markers accounting for transcriptional activity. This seemed all the more relevant, since investigations based on DNA did not point to the specificities that were observed using RNA-based techniques.

This study, based on 16S rRNA and 16S rRNA gene comparison, showed that not all fecal bacteria of patients with UC have the same transcriptional activity and that the biodiversity of the active microbiota is lower for UC patients than for healthy controls. Interestingly, an active *E. coli* (or related enterobacteria) was significantly associated with UC. Although the number of samples investigated could seem low, this result was statistically significant.

TTGE separates bacterial DNA fragments with similar sizes but different levels of thermal stability (22, 31). Sequences differing by a single base can be separated by this method. Applied to complex microbial communities, TTGE yields profiles corresponding to all the dominant bacterial species present in the sample. DNA patterns reflect the dominant bacterial diversity of the fecal microbiota. However, these methods do not distinguish dead bacteria from bacteria with low metabolic activity or from "transcriptionally active" bacteria. In contrast, analysis of rRNA detects only active bacteria. In our work, TTGE allowed discrimination of fewer than 20 bands in each sample, whereas sequencing clones from 16S rRNA gene libraries could give a better resolution of the composition of fecal microbiota. Nevertheless, the latter technique is still limited in terms of throughput, while TTGE is a quite powerful tool for the comparative assessment of dominant intestinal microbiota from numerous individuals. Indeed, our observations confirm that TTGE is appropriate for identifying specific traits of the dominant intestinal microbiota when comparing nutritional or pathological conditions, and they further emphasize the relevance of using RNA as a matrix rather than DNA.

As mentioned in Table 2, the best match obtained for our sequence was *E. coli* and some other enterobacteria (*Escherichia albertii*, *Escherichia fergusonii*, *Shigella boydii*, *Shigella flexneri*, *Salmonella enterica* serovar Typhi, and *Photorhabdus luminescens*). Except for the last one, which corresponds to entomopathogenic bacteria, all these bacteria are pathogenic and lead to infectious colitis in humans (1, 6, 8, 9). All patients in the present study underwent repeated stool cultures, and their clinical situation improved when they were treated (after the fecal sampling) by corticosteroids, which further suggests that they did not suffer from infectious colitis. Taken together, these data suggest that our band of interest represents nonpathogenic enterobacteria. *Escherichia coli* seemed the best candidate to us.

TTGE DNA profiles may be influenced by the number of rRNA operons in a given bacterial species. The number of rRNA genes ranges from 1 to as many as 15 copies (11). For example, *E. coli* possesses 7 rRNA operons (30), whereas *Clostridium perfringens* has 10 (23). A bacterium with a large number of rRNA operons might yield a more intense band on TTGE DNA gels. In contrast, TTGE RNA profiles should not be influenced by the number of rRNA operons but rather by the rRNA content, which can vary from 1,000 to 100,000 ribosomes in *E. coli*, for example. Bacteria containing the largest number of ribosomes and, consequently, the largest number of rRNA sequences are the most metabolically active. Zoetendal et al. (31) used this method to analyze fecal samples from two healthy subjects. They observed, as confirmed in our study, that some bands were more prominent in the TTGE RNA profiles than in the TTGE DNA profiles and concluded that not all bacteria of the fecal microbiota have the same metabolic activity. Thus, some dominant bacteria have low transcriptional activity while some subdominant bacteria can have high transcriptional activity. This is not specific to UC patients, since healthy controls show the same differences. However, we observed a reduction in the biodiversity of the active portion of the fecal microbiota in UC patients relative to healthy controls. Restricted biodiversity has also been observed in this setting by Ott et al., using single-strand conformation polymorphism (18).

In a recent work using fluorescence in situ hybridization analysis, we compared the phylogenetic group composition of fecal microbiota between UC and healthy subjects (23a). This study indicated significant differences, but restricted to *Firmicutes*, notably a decrease in the proportion of bacteria from the *Clostridium coccoides* phylogenetic group. The proportion of enterobacteria did not differ between UC and healthy subjects. This fluorescence in situ hybridization-based study allowed detection of the presence of bacteria independently of their transcriptional activity, unlike the rRNA-based TTGE approach chosen in the present work. This could thus suggest that

enterobacteria may not be overrepresented in the UC fecal microbiota but that they may be particularly metabolically active (with high RNA contents).

Previous studies have shown alterations in the UC microbiota, involving *Bacteroides vulgatus* (16), sulfate-reducing bacteria (19), and several *Enterobacteriaceae* (17, 28). However, these studies did not take bacterial metabolic activity into account.

Several lines of evidence implicate *E. coli* dysbiosis in UC. Giaffer et al. isolated adhesive *E. coli* in feces from 68% of patients with UC, compared to only 6% of healthy controls (7). Likewise, an original pathovar of *E. coli* with entero-adhesive properties was found to be more abundant in ileal lesions of patients with Crohn's disease than in controls (3, 4). Whether these abnormalities are a cause or an effect of gut wall inflammation or mucus alteration remains to be determined. In our study, interindividual comparison of the active fecal microbiota showed reduced biodiversity and also an rRNA sequence corresponding to that of *E. coli* for eight of the nine UC patients. Moreover, intraindividual comparisons showed that one of six sequenced bands that were present in the RNA profile but not in the corresponding TTGE DNA profile of UC patients corresponded to that of *E. coli*.

Interestingly, randomized controlled trials have shown that the probiotic *E. coli* strain Nissle 1917, which antagonizes the growth of other *E. coli* strains, is as efficient as mesalazine in preventing recurrences of UC (12). *E. coli* Nissle 1917 might act by competing with detrimental endogenous *E. coli* strains (10). Other studies have shown that *E. coli* Nissle 1917 induces defensin expression in intestinal cell lines, and this may also suppress endogenous *E. coli* (29). Finally, it has been shown that perinuclear antineutrophilic cytoplasmic antibodies, which are found in 60 to 90% of UC patients (20), target a recurrent protein epitope expressed by *E. coli* and *Bacteroides caccae* (2).

In conclusion, this study shows that the biodiversity of active bacteria in the dominant fecal microbiota of UC patients is lower than that of healthy subjects and that *E. coli* is overrepresented in UC patients' active microbiota. These findings further support the suspected role of *E. coli* in the onset and/or chronicity of IBD. Additional studies should also assess this during remission and within the mucosa-associated microbiota, which differs from the luminal microbiota and is in close proximity to the epithelial and immune cells.

ACKNOWLEDGMENT

H. Sokol received the Fondation pour la Recherche Medicale Fellowship Grant to perform this work.

REFERENCES

1. **Chaudhury, A., G. Nath, A. Tikoo, and S. C. Sanyal.** 1999. Enteropathogenicity and antimicrobial susceptibility of new Escherichia spp. J. Diarrhoeal Dis. Res. **17:**85–87.
2. **Cohavy, O., D. Bruckner, L. K. Gordon, R. Misra, B. Wei, M. E. Eggena, S. R. Targan, and J. Braun.** 2000. Colonic bacteria express an ulcerative colitis pANCA-related protein epitope. Infect. Immun. **68:**1542–1548.
3. **Darfeuille-Michaud, A., J. Boudeau, P. Bulois, C. Neut, A. L. Glasser, N. Barnich, M. A. Bringer, A. Swidsinski, L. Beaugerie, and J. F. Colombel.** 2004. High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. Gastroenterology **127:**412–421.
4. **Darfeuille-Michaud, A., C. Neut, N. Barnich, E. Lederman, P. Di Martino, P. Desreumaux, L. Gambiez, B. Joly, A. Cortot, and J. F. Colombel.** 1998. Presence of adherent Escherichia coli strains in ileal mucosa of patients with Crohn's disease. Gastroenterology **115:**1405–1413.
5. **Dore, J., G. Gramet, I. Goderel, and P. Pochart.** 1998. Culture-independent characterisation of human faecal flora using rRNA-targeted hybridisation probes. Genet. Sel. Evol. **4**(Suppl. 1)**:**S287–S296.
6. **Farmer, J. J., III, G. R. Fanning, B. R. Davis, C. M. O'Hara, C. Riddle, F. W. Hickman-Brenner, M. A. Asbury, V. A. Lowery III, and D. J. Brenner.** 1985. *Escherichia fergusonii* and *Enterobacter taylorae*, two new species of *Enterobacteriaceae* isolated from clinical specimens. J. Clin. Microbiol. **21:**77–81.
7. **Giaffer, M. H., C. D. Holdsworth, and B. I. Duerden.** 1992. Virulence properties of Escherichia coli strains isolated from patients with inflammatory bowel disease. Gut **33:**646–650.
8. **Huys, G., M. Cnockaert, J. M. Janda, and J. Swings.** 2003. Escherichia albertii sp. nov., a diarrhoeagenic species isolated from stool specimens of Bangladeshi children. Int. J. Syst. Evol. Microbiol. **53:**807–810.
9. **Hyma, K. E., D. W. Lacher, A. M. Nelson, A. C. Bumbaugh, J. M. Janda, N. A. Strockbine, V. B. Young, and T. S. Whittam.** 2005. Evolutionary genetics of a new pathogenic *Escherichia* species: *Escherichia albertii* and related *Shigella boydii* strains. J. Bacteriol. **187:**619–628.
10. **Ingrassia, I., A. Leplingard, and A. Darfeuille-Michaud.** 2004. Lactobacillus casei DN-114 001 inhibits adhesion to and invasion of intestinal epithelial cells by adherent-invasive E. coli isolated from Crohn's disease. Gastroenterology **126**(Suppl. 2)**:**A579.
11. **Klappenbach, J. A., P. R. Saxman, J. R. Cole, and T. M. Schmidt.** 2001. rrndb: the Ribosomal RNA Operon Copy Number Database. Nucleic Acids Res. **29:**181–184.
12. **Kruis, W., P. Fric, J. Pokrotnieks, M. Lukas, B. Fixa, M. Kascak, M. A. Kamm, J. Weismueller, C. Beglinger, M. Stolte, C. Wolff, and J. Schulze.** 2004. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. Gut **53:** 1617–1623.
13. **Lepage, P., P. Seksik, M. de la Cochetière, L. Rigottier-Gois, R. Jian, P. Marteau, and J. Dore.** 2003. Molecular study of the ileal and colonic mucosa associated microbiota in IBD. Gastroenterology **124**(Suppl. 1)**:**T1066.
14. **Lepage, P., P. Seksik, M. Sutren, M. F. de la Cochetiere, R. Jian, P. Marteau, and J. Dore.** 2005. Biodiversity of the mucosa-associated microbiota is stable along the distal digestive tract in healthy individuals and patients with IBD. Inflamm. Bowel Dis. **11:**473–480.
15. **Marteau, P., P. Pochart, J. Dore, C. Bera-Maillet, A. Bernalier, and G. Corthier.** 2001. Comparative study of bacterial groups within the human cecal and fecal microbiota. Appl. Environ. Microbiol. **67:**4939–4942.
16. **Matsuda, H., Y. Fujiyama, A. Andoh, T. Ushijima, T. Kajinami, and T. Bamba.** 2000. Characterization of antibody responses against rectal mucosa-associated bacterial flora in patients with ulcerative colitis. J. Gastroenterol. Hepatol. **15:**61–68.
17. **Mylonaki, M., N. B. Rayment, D. S. Rampton, B. N. Hudspith, and J. Brostoff.** 2005. Molecular characterization of rectal mucosa-associated bacterial flora in inflammatory bowel disease. Inflamm. Bowel Dis. **11:**481–487.
18. **Ott, S. J., M. Musfeldt, D. F. Wenderoth, J. Hampe, O. Brant, U. R. Folsch, K. N. Timmis, and S. Schreiber.** 2004. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. Gut **53:**685–693.
19. **Pitcher, M. C., E. R. Beatty, and J. H. Cummings.** 2000. The contribution of sulphate reducing bacteria and 5-aminosalicylic acid to faecal sulphide in patients with ulcerative colitis. Gut **46:**64–72.
20. **Quinton, J. F., B. Sendid, D. Reumaux, P. Duthilleul, A. Cortot, B. Grandbastien, G. Charrier, S. R. Targan, J. F. Colombel, and D. Poulain.** 1998. Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. Gut **42:**788–791.
21. **Sartor, R. B.** 2004. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. Gastroenterology **126:**1620–1633.
22. **Seksik, P., L. Rigottier-Gois, G. Gramet, M. Sutren, P. Pochart, P. Marteau, R. Jian, and J. Dore.** 2003. Alterations of the dominant faecal bacterial groups in patients with Crohn's disease of the colon. Gut **52:**237–242.
23. **Shimizu, T., K. Ohtani, H. Hirakawa, K. Ohshima, A. Yamashita, T. Shiba, N. Ogasawara, M. Hattori, S. Kuhara, and H. Hayashi.** 2002. Complete genome sequence of Clostridium perfringens, an anaerobic flesh-eater. Proc. Natl. Acad. Sci. USA **99:**996–1001.

23a. **Sokol, H., P. Seksik, L. Rigottier-Gois, C. Lay, P. Lepage, I. Podglajen, P. Marteau, and J. Dore.** 2006. Specificities of the fecal microbiota in inflammatory bowel disease. Inflamm. Bowel Dis. **12:**106–111.

24. **Suau, A., R. Bonnet, M. Sutren, J. J. Godon, G. R. Gibson, M. D. Collins, and J. Dore.** 1999. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. Appl. Environ. Microbiol. **65:**4799–4807.
25. **Sutren, M., M. Michel, M. de la Cochetière, A. Bernalier, D. Wils, M. Saniez, and J. Dore.** 1998. Temporal temperature gradient gel electrophoresis (TTGE) is an appropriate tool to assess dynamics of species diversity of the human fecal flora. Reprod. Nutr. Dev. **40:**176–177.
26. **Swidsinski, A., A. Ladhoff, A. Pernthaler, S. Swidsinski, V. Loening-Baucke, M. Ortner, J. Weber, U. Hoffmann, S. Schreiber, M. Dietel, and H. Lochs.** 2002. Mucosal flora in inflammatory bowel disease. Gastroenterology **122:** 44–54.

27. **Tamboli, C. P., C. Neut, P. Desreumaux, and J. F. Colombel.** 2004. Dysbiosis in inflammatory bowel disease. Gut **53:**1–4.
28. **von Wulffen, H., H. Russmann, H. Karch, T. Meyer, M. Bitzan, T. C. Kohrt, and S. Aleksic.** 1989. Verocytotoxin-producing Escherichia coli O2:H5 isolated from patients with ulcerative colitis. Lancet **i:**1449–1450.
29. **Wehkamp, J., J. Harder, K. Wehkamp, B. Wehkamp-von Meissner, M. Schlee, C. Enders, U. Sonnenborn, S. Nuding, S. Bengmark, K. Fellermann, J. M. Schroder, and E. F. Stange.** 2004. NF-kappaB- and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by *Escherichia coli* Nissle 1917: a novel effect of a probiotic bacterium. Infect. Immun. **72:**5750–5758.
30. **Welch, R. A., V. Burland, G. Plunkett III, P. Redford, P. Roesch, D. Rasko, E. L. Buckles, S. R. Liou, A. Boutin, J. Hackett, D. Stroud, G. F. Mayhew, D. J. Rose, S. Zhou, D. C. Schwartz, N. T. Perna, H. L. Mobley, M. S. Donnenberg, and F. R. Blattner.** 2002. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic Escherichia coli. Proc. Natl. Acad. Sci. USA **99:**17020–17024.
31. **Zoetendal, E. G., A. D. Akkermans, and W. M. De Vos.** 1998. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. Appl. Environ. Microbiol. **64:**3854–3859.