Transcriptional activity of the dominant gut mucosal microbiota in chronic inflammatory bowel disease patients

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Dysbiosis of the gut mucosa-associated microbiota (MAM) plays a pivotal role in the pathogenesis of chronic inflammatory bowel diseases (IBD). To date, dysbiosis only describes the altered composition of the different bacterial populations, but little is known about transcriptional activity, metabolism and the 'live' status of the MAM. In this study we investigated the transcriptional activity of the dominant intestinal bacterial populations in patients with IBD. Colonic mucosal biopsies from patients with active Crohn's disease (CD; $n=10$), active ulcerative colitis (UC; $n=10$) and healthy individuals (HI; $n=10$) were compared by 16S rRNA gene and rRNA profiles using clone libraries with more than 1700 sequenced clones. Bacterial richness was significantly lower in clone libraries based on rRNA compared to those based on the rRNA genes in the CD group (3.01 vs 3.91) and the UC group (3.61 vs 4.15), but showed no difference in HI (3.81 vs 3.85). The qualitative composition of rRNA and rRNA gene clone libraries was significantly different, with the phylum *Bacteroidetes* being the most active ($P<0.01$) compared to other populations in all clinical groups. In contrast, *Actinobacteria* and *Firmicutes* were inactive in the CD group, while *Escherichia* sp. were both abundant and active in the CD and UC groups. Most of the phylotypes showing the highest activity index ratios represented less than 1 % of the microbiota. Our findings indicate that specific bacterial populations are activated in IBD patients, while other groups are in an inactive or 'dormant' state. The transcriptional activity points to a more functional role of the intestinal mucosal microbiota and may lead to the identification of therapeutic targets in the active modulation of microbial factors.

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INTRODUCTION

Chronic inflammatory bowel diseases (IBD) are characterized by dysbiosis of the intestinal microbiota (Ott *et al.*, 2004; Tamboli *et al.*, 2004; Manichanh *et al.*, 2006). Dysbiosis is discussed as a major factor in disease pathogenesis along with genetic mutations and a dysregulation of the immune response to bacterial antigens (Schreiber *et al.*, 2005). Loss of natural intestinal diversity and a shift of bacterial composition towards a more deleterious profile might reflect the net effect of environmental influences

over the past few decades leading to the dramatic increase of the incidence of IBD in the industrialized world (Ott *et al.*, 2004, 2008a; b; Manichanh *et al.*, 2006). To date, dysbiosis only describes the altered composition of the different bacterial populations, but little is known about transcriptional activity, metabolism and 'live' status of the mucosa-associated microbiota (MAM), which may have more relevance for the host, e.g. by direct contact with the gut epithelium, secretion of pro-inflammatory cytokines, quorum-sensing molecules or other microbe-associated molecular patterns.

A maximum of 10–30 % of the total microbes of the complex bacterial ecosystem within the gut can be cultivated using classical microbiological approaches (McFarlane & Gibson, 1994; Langendijk *et al.*, 1995; Wilson & Blitchington, 1996; Hugenholtz *et al.*, 1998; Suau *et al.*, 1999; Tannock, 2001, 2002). New cultivation-independent techniques based on ribosomal marker genes

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Abbreviations: AIR, activity index ratio; CAI, clinical activity index; CD, Crohn's disease; CDAL, Crohn's disease activity index; HI, healthy individuals; IBD, inflammatory bowel disease; MAM, mucosa-associated microbiota; OTU, operational taxonomic unit; UC, ulcerative colitis.

A supplementary table of phylotype information is available as supplementary material with the online version of this paper.

(rRNA genes/rRNA) have facilitated the understanding of the composition of microbial communities (McFarlane & Gibson, 1994; Langendijk *et al.*, 1995; Wilson & Blitchington, 1996; Hugenholtz *et al.*, 1998; Tannock, 2001, 2002). Analysis of the 16S rRNA gene has previously been targeted to describe bacterial variability of the intestinal MAM in IBD patients and healthy individuals (HI) (Poxton *et al.*, 1997; Zoetendal *et al.*, 2002; Lepage *et al.*, 2005; Seksik *et al.*, 2005; Gophna *et al.*, 2006). The taxonomic and phylogenetic information obtained by the 16S rRNA gene approach reflects the global composition of a microbial community, but contains no further characterization on the transcriptional status and 'activity' of the intestinal microbiota. The transcriptional status of bacteria is represented by expression of mRNA equivalents, thus detection of bacterial rRNA correlates with metabolic activity. Two works identified active faecal bacteria in HI and patients with IBD (Zoetendal *et al.*, 1998; Sokol *et al.*, 2006) using rRNA-based fingerprinting approaches, yet faecal microbiota differs from the MAM (Lepage *et al.*, 2005; Eckburg *et al.*, 2005).

In the present study diversity and transcriptional activity of the intestinal MAM was investigated in patients with Crohn's disease (CD), ulcerative colitis (UC) and HI by comparison of their rRNA and rRNA gene sequences in order to dissect the metabolic status from the mere presence of intestinal microbiota and to gain more functional information about active components of the MAM in IBD patients.

METHODS

Patients. Ten patients with CD, ten patients with UC and ten HI were included in this study. All participants were of white ethnicity. The diagnosis of IBD was made in accordance with established clinical, endoscopic, histological and radiological criteria (Ott *et al.*, 2004). Disease activity was assessed by the CD activity index (CDAI) for CD patients and the clinical activity index (CAI) for UC patients. All IBD patients were in an active phase of the disease (CDAI >150 in the CD group and CAI >4 in the UC group). The demographic data for the patients enrolled are shown in Table 1. Biopsies were sampled from macroscopic sites of inflammation in the sigmoid colon. To assess inflammatory activity, histological parameters were determined for IBD patients by a pathologist following the criteria described by Geboes & Dalle (2002) and Geboes *et al.* (2000). The control group included HI (from screening colonoscopies without pathological macroscopic or histological findings). Inclusion criteria were that no antibiotic therapy had been administered at the time of investigation and in the last 6 months. Patient interviews about their nutritional habits revealed neither a significant difference in the uptake of food containing fungal components (e.g. cheese) nor a significant difference in the use of parenteral or formula diets.

DNA and RNA extraction. DNA and RNA were extracted simultaneously from individual biopsies using the AllPrep DNA/RNA mini kit (Qiagen) according to the manufacturer's instructions. The concentration and purity of DNA and RNA was assessed photometrically. To check for DNA contamination in the RNA preparations, PCR was performed using the primers GADPH_F2 and GADPH_R2 amplifying 334 bases from the glyceraldehyde-3-phosphate dehydrogenase-encoding gene.

Table 1. Description of human subjects

	HI group	CD group	UC group
Gender: male/ female	3/7	4/6	5/5
Median age (years)	70.1	35.1	45.3
Treatment			
Corticosteroids	—	2	—
NSAID	—	—	—
Purine analogues	—	—	—
5-ASA	—	—	1
Biologicals	—	—	—
Specific disease activity	—	CDAI >150	CAI >4

5-ASA, 5-Aminosalicylic acid (mesalazine); NSAID, non-steroidal anti-inflammatory drug.

Reverse transcription of RNA. Reverse transcription was performed using MMLV reverse transcriptase (Promega). A mixture of 0.5 µg total RNA, 1 µl random primers (Promega) and 1 µl RNase inhibitor (Promega) was incubated for 5 min in a total volume of 10 µl at 70 °C and cooled on ice. After adding 4 µl MMLV 5 × reaction buffer (Promega), 20 mM dNTPs and, finally, 1 µl MMLV reverse transcriptase in a total volume of 20 µl, the reaction mixture was incubated for 2 h at 42 °C.

PCR amplification of 16S rRNA and rRNA genes. A selected region of the 16S rRNA gene, including the variable regions V4 and V5 (*Escherichia coli* reference positions 519–926), was amplified separately from each individual with the universal bacterial primers Com1 and Com2 as previously described (Ott *et al.*, 2004). PCR products were analysed by 1.5% agarose gel electrophoresis to ascertain amplicon size (about 400 bp).

Cloning and sequencing. Amplicons were purified with a MinElute PCR purification kit (Qiagen). The same amount of rRNA gene and rRNA PCR products from the same clinical group were pooled and cloned into competent *Escherichia coli* cells using the PCR 2.1 TOPO TA cloning kit (Invitrogen). Six clone libraries were constructed (both rRNA and rRNA gene for each clinical group) containing 288 clones each. Inserts were amplified using the vector specific primers M13F and M24R as described previously (Ott *et al.*, 2004). Sequencing of inserts was performed with an ABI Prism 3700 DNA analyser in a final volume of 10 µl using 1 µl ABI Prism BigDye (Applied Biosystems) at a concentration of 3.2 µM vector-specific primers according to the following protocol: 96 °C for 1 min; 25 cycles of 96 °C for 10 s, 53 °C for 5 s and 60 °C for 4 min.

Sequence analysis and phylotype determination. The resultant sequences were checked for possible chimeric artefacts by the chimera check program (Ribosomal Database Project II, RDP II– <http://rdp.cme.msu.edu>) (Cole *et al.*, 2003). For phylogenetic affiliation, sequences from rRNA and rRNA gene libraries were subjected to National Center for Biotechnology Information BLAST analysis (<http://www.ncbi.nlm.nih.gov/BLAST>) and the sequences match and classifier program at RDP II (<http://rdp.cme.msu.edu/>) (Cole *et al.*, 2007, 2009). Sequences were aligned using the CLUSTAL_X program and alignment was refined with BioEdit software (version 7.0.5.3). Distance matrices for each library were calculated with the DNADIST

program, which is part of the PHYLIP software package, according to the Jukes–Cantor model. Sequences showing at least 97 % similarity were clustered into species level (Schloss & Handelsman, 2004) phylotype or OTU (operational taxonomic unit) using the DOTUR software (distance-based OTU and richness) (Schloss & Handelsman, 2005). Additionally, the ARB database (<http://www.arb-home.de>) was used for ribosomal sequences and sequence handling; the VecScreen database (<http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html>) was also used.

Statistical analysis and comparison of 16S clone libraries.

Good's coverage estimation was calculated as $[1 - (n/N)] \times 100$, where n is the number of singleton sequences and N is the total number of sequences within the analysed clone library. Estimates of phylotype richness were calculated according to the abundance-based coverage estimator (ACE) (Chao & Lee, 1992) and the bias-corrected Chao1 estimator (Chao, 1984). Collector's curves of observed and estimated richness were calculated using DOTUR (Schloss & Handelsman, 2005).

A principal component analysis was performed to document the distances between phylotype composition and quantity among rRNA and rRNA gene libraries, generated from HI, CD patients and UC patients. Phylotype constitution similarities among clinical groups were compared by using the Sorensen index, $C_s = 2J/(a+b)$, where J is the number of common shared phylotypes found in both libraries (i.e. A and B) subjected to comparison, a is the total number of phylotypes in library A and b is the total number of phylotypes in library B. To determine if a phylotype is active or dormant, activity index ratios (AIRs) at different taxonomical levels were calculated (AIR = percentage of sequences in rRNA library/percentage of sequences in rRNA gene library).

Ethical considerations. The protocol was approved by the review board of the Medical Faculty of the Christian Albrechts University (CAU) in Kiel prior to use. All patients gave written informed consent prior to colonoscopy.

RESULTS

A total of 1728 clones were sequenced using vector-specific primers and 1571 were considered for further analysis (after exclusion of chimera, eukaryotic sequences and low quality sequences). Clones with ≥ 97 % sequence similarity were grouped into phylotypes according to Schloss &

Handelsman (2004). The numbers of clones and phylotypes analysed in the different groups are presented in Table 2. The Good's coverage estimates ranged from 71.36 % in UC rRNA gene library to 83.26 % in CD rRNA, indicating the good quality of the libraries and sufficient representation of diversity (Table 2). Among the rRNA gene libraries we observed a reduced number of phylotypes in CD patients ($n=79$) and an increased number in UC patients ($n=101$) compared to controls ($n=87$). The direct comparison of rRNA gene and rRNA libraries revealed equal numbers of phylotypes among the HI ($n=87$ each), while the number of phylotypes in CD and UC patients was higher in rRNA gene libraries compared to the corresponding rRNA libraries indicating a discrepancy between the diversity of transcriptionally active bacteria (rRNA) and bacteria present (rRNA gene) in IBD patients. Likewise, principal component analysis of phylotypes from the different clone libraries showed that variation along the first two principal axes was influenced by the clinical status and type of nucleic acid used to generate clone libraries. Libraries from rRNA or rRNA gene seemed to be similar in HI, whereas rRNA and rRNA gene libraries differed in both CD and UC groups (Fig. 1). Furthermore, rRNA and rRNA gene libraries from HI shared more common phylotypes (Sorensen similarity index 0.609) compared with CD patients (Sorensen similarity index 0.434) and UC patients (Sorensen similarity index 0.427). A small part of phylotypes had no representation in rRNA gene libraries but was present in rRNA clone library and vice versa. It was noteworthy that bacterial phylotypes activity was not linked to their prevalence in the rRNA gene libraries. Within the three clinical groups, most of the phylotypes showing the highest AIRs represented less than 1 % of the microbiota (Fig. 2).

Regardless of the clinical status or type of nucleic acid (rRNA or rRNA gene), phylotypes clustered into the four main phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*. The percentage representations of sequences in each phylum are shown in Table 3.

Table 2. Estimated statistical indicators of 16S rRNA genes and rRNA clone libraries generated from HI and patients with CD and UC

	HI group		CD group		UC group	
	rRNA	rRNA gene	rRNA	rRNA gene	rRNA	rRNA gene
No. of sequences	255	271	257	224	277	227
Phylotypes (OTUs)	87	87	70	79	85	101
Singletons	52	55	43	43	49	65
Chao1 estimator of species richness	219.60	210.75	160.30	161.09	163.40	239.67
ACE	186.304	244.144	175.669	144.840	174.940	264.857
Evenness	0.69	0.68	0.59	0.72	0.64	0.75
Good estimator of coverage	79.60	79.70	83.26	80.80	82.31	71.36

ACE, Abundance-based coverage estimator.

Fig. 1. Principal component analysis of libraries from CD and UC patients and HI. The percentages of sequences of each phylotype were subjected to principal component analysis. Clone libraries generated from rRNA genes (○, □, △) and rRNA (●, ■, ▲) were plotted against the first two principal components. Principal component analysis of phylotypes from the different clone libraries showed that variation along the first two principal axes was influenced by the clinical status and type of nucleic acid used to generate clone libraries. Libraries from rRNA or rRNA gene seemed to be similar in HI, whereas rRNA and rRNA genes libraries differed in both CD and UC groups.

Firmicutes are less active in CD patients

The prevalence of bacteria from the phylum *Firmicutes* in rRNA gene libraries was significantly lower in the IBD group compared to controls (52.67 % in the CD group and 59.47 % in the UC group compared to 78.59 % in the

HI group ($P=0.01$) (Table 3). The decreased prevalence of this phylum in the gene libraries was also observed in the rRNA libraries (37.35 % in CD patients and 48.01 % in UC patients compared to 68.62 % in HI). Interestingly, diversity within the phylum *Firmicutes* was only reduced in CD patients compared to HI with a total number of 53 and 68 phylotypes, respectively, whereas diversity was conserved in UC patients (phylotypes=71). In HI, nearly half of the phylotypes belonging to the *Firmicutes* were active (47.0 %). This percentage decreased dramatically in IBD patients (9.4 % for CD and 15.9 % for UC groups).

Phylotypes with significant number of clones and high AIRs in HI libraries were related to species *Catenibacterium mitsuokai*, *Clostridium innocuum* and *Clostridium ramosum*, whereas phylotypes related to *Clostridium leptum*, *Dorea longicatena* and *Eubacterium bifforme* were less active in HI (Fig. 3). *Ruminococcus gnavus* and related phylotypes were found to be active in HI (AIR=2.217) and CD patients, while they showed less activity in UC patients (AIR=0.27). In contrast, *Ruminococcus lactaris* and related phylotypes showed low activity in HI and UC patients, but were active in CD patients. In the CD group, phylotypes with high activity ratios belonged to: *Clostridium ramosum* and *Eubacterium ramulus*. However, phylotypes showing low AIRs in the CD group were related to bacterium mpn-isolate group 18, genus *Lachnospiraceae incertae sedis*, *Faecalibacterium prausnitzii* L2-6, *Roseburia intestinalis* L1-82 and *Eubacterium bifforme*.

Four butyrate-producing bacteria SL6/1/1 related phylotypes were found to be active members of the intestinal community in UC patients. Similarly, *Ruminococcus obeum* 1-33 related phylotypes were also found to be more active in UC patients compared with HI and CD patients (Fig. 3). The complete list of phylotypes is available in Supplementary Table S1 (available with the online journal).

Fig. 2. AIRs of each phylotype as a function of their abundance for each clinical group. The AIRs are shown on the y axis of the graph against the prevalence of phylotypes in the overall rRNA gene libraries (%). This figure demonstrates that bacterial phylotype activity was not linked to their prevalence in the rRNA gene libraries. Within the three clinical groups, most of the phylotypes showing the highest AIRs represented less than 1 % of the microbiota. ■, CD group; ▲, UC group; ●, HI group.

Table 3. Percentage representation of sequences among different phyla in IBD patients and HI (controls)

	HI group		CD group		UC group	
	rRNA	rRNA gene	rRNA	rRNA gene	rRNA	rRNA gene
Firmicutes	68.63	78.59	37.35	52.68	48.01	59.47
Bacteroidetes	19.61	8.85	46.70	23.21	27.07	22.47
Proteobacteria	8.23	5.16	12.45	11.16	20.93	12.34
Actinobacteria	3.53	6.64	3.50	12.95	3.24	2.64
Other	—	0.76	—	—	0.72	3.08

Bacteroides are prevalent and active in CD patients

Bacteroidetes was the second most dominant major phylum in all libraries, representing 23.21 % of phylotypes in the CD group, 22.46 % in the UC group and 8.85 % in the HI group at the rRNA gene level. The mean AIRs were the highest in HI (AIR=2.21), followed by the CD group (2.01) and the UC group (1.20).

Bacteroides fragilis-related phylotypes were less represented in CD patients compared to HI or UC patients, but AIR of this species was the highest in CD patients (AIR=10.46 in CD vs 2.87 in HI and 1.31 in UC). Likewise, phylotypes

related to *Bacteroides vulgatus* constituted active members in CD patients compared to HI or UC patients and was also the overall most active phylotype in the CD group with an AIR of 53.04 (Fig. 3). Interestingly, three phylotypes, close relatives of *Bacteroides eggerthii* were present only in the CD rRNA gene library indicating dormant behaviour of this species in the CD group (see Supplementary Table S1 available with the online journal). *Bacteroides* sp. AR20 and *Bacteroides dorei*-related phylotypes were specifically observed in libraries generated from UC individuals, with *Bacteroides* sp. AR20 showing a dormant/inactive status (AIRs below 1) while the latter was active (Fig. 3).

Fig. 3. Level of transcriptional activity among noticeable species. AIRs were calculated as percentage representations of clones in the rRNA library divided by the percentage representation of clones in the rRNA gene library. Black bars, CD patients; grey bars, UC patients; white bars, HI.

***Escherichia coli* is prevalent and active in UC patients**

Phylotypes related to *Escherichia coli* were detected in all libraries, with a higher prevalence in UC patients (10.57 %) compared to CD patients (7.59 %) and HI (0.37 %). However, *Escherichia coli* phylotypes were active in all three clinical groups with AIR ranging from 1.68 in the CD group to 3.19 in the HI group (Fig. 3, Supplementary Table S1 available with the online journal).

Actinobacteria are less active in CD patients and more active in UC patients

Actinobacteria (high G + C content Gram-positive bacteria) of the genera *Bifidobacteria*, *Eggerthella* and *Collinsella* were detected in all three clinical groups. The overall prevalence of this phylum was increased in CD patients (12.94 % of the total microbiota) in comparison to HI (6.64 %) and UC patients (2.64 %) (Table 3). Dominant bacteria from this phylum in CD patients were *Collinsella aerofaciens* and *Eggerthella* genus. However, the only active bacteria within the *Actinobacteria* was related to *Bifidobacterium bifidum* and was only observed in UC patients (AIR=4.09).

DISCUSSION

Dysbiosis of the mucosa-associated intestinal microbiota is one of the major characteristics in patients with chronic IBD. Previous microbiological studies have produced conflicting results about the nature of dysbiosis in IBD patients and the contribution to disease pathogenesis and pathophysiology (Ott *et al.*, 2004, 2008a, b; Manichanh *et al.*, 2006; Gophna *et al.*, 2006; Lucke *et al.*, 2006; Frank *et al.*, 2007). Generally, dysbiosis seems to be independent of whether the sample originated from inflamed or non-inflamed sites (Vasquez *et al.*, 2007). New molecular techniques based on the 16S rRNA gene allowed fresh insights into the composition of the complex intestinal microbiota and their alterations in inflammatory gastrointestinal disorders. However, the detection of 16S rRNA gene signatures provides important information about the presence of bacteria, but does not predict the functional status of micro-organisms. The ratio between 16S rRNA and 16S rRNA genes is a predictor of the metabolic status of bacteria in a microbial community, and allows the differentiation between active and inactive members of the intestinal microbiota (Wagner, 1994). A higher transcriptional activity corresponds to a higher cell replication rate and greater protein production. Therefore, the lack of an exact definition of the dysbiotic status of the IBD microbiota might be attributed to the unidirectional view on micro-organisms by a DNA-based approach.

Fingerprinting techniques and fluorescence *in situ* hybridization combined with flow cytometry have previously been implicated to detect metabolically active bacteria in faecal samples of HI and patients with IBD (Zoetendal

et al., 1998; Sokol *et al.*, 2006; Rigottier-Gois *et al.*, 2003). In UC patients, Sokol *et al.* (2006) observed an active *Escherichia coli* (or related *Enterobacteria*) significantly associated with faeces from UC patients compared to HI. In faecal samples of 23 HI, significant differences were found for *Bacteroides* and *Atopobium* comparing 16S rRNA and 16S rRNA genes (Rigottier-Gois *et al.*, 2003). In our study, the prevalence of *Escherichia coli* was highlighted at the mucosal level in IBD patients, mostly in UC patients. If the AIR of *Escherichia coli* in HI was higher than in IBD patients, the corresponding phylotype accounted for only 0.37 % of the total microbiota in the HI group compared to 9.25 % in the UC group. Thus, *Escherichia coli* is also an active species of the MAM in UC patients. This bacterial species has already been implicated in IBD dysbiosis (Giaffer *et al.* 1992; Darfeuille-Michaud *et al.*, 1998).

The overall diversity on the rRNA gene level revealed a decreased diversity in CD patients compared to controls as reported previously (Ott *et al.*, 2004). This was mainly due to a lower diversity within the phylum *Firmicutes*, in line with a previous study based on metagenomic analysis of the faecal microbiota in CD patients (Manichanh *et al.*, 2006). Interestingly, bacterial phylotype numbers in UC patients were increased compared to the HI group. Previously our group demonstrated a reduced diversity in UC patients (Ott *et al.*, 2004), also by applying an electrophoretic approach (generally able to distinguish dominant and abundant microbiota), differences between studies might be due to different methodological approaches: in contrast to the electrophoretic approach, the clone library methodology used in the current study could also pick under-represented bacterial strains/species. A baseline analysis of the relative proportions of rRNA and rRNA gene sequences revealed a balanced ratio in the controls, but showed a significant lack of transcriptional activity in IBD patients. If the bacterial diversity of *Firmicutes* was low only in CD patients, activity of bacteria from this phylum was dramatically decreased in both IBD groups compared to HI. Phylotypes uniquely active in HI were mainly related to the family *Lachnospiraceae* (63 %) or the family *Erysipelotrichaceae* (27 %). This might suggest a key role of these bacteria in homeostasis maintenance, or a stronger vulnerability toward inflammatory response.

The prevalence of *Bacteroides* sp. in IBD patients is in agreement with other studies (Swidsinski *et al.*, 2002; Hartley *et al.*, 1992). *Bacteroides* sp. produce mucin-degrading sulfatases that can lead to impairment of the barrier function of the epithelial cell layer in IBD patients (Lucke *et al.*, 2006). Swidsinski *et al.* (2002) analysed gut microbiota mucosal biofilms in inflammatory and healthy volunteers using fluorescent *in situ* hybridization. As this method, also based on the 16S rRNA molecule, is targeting the rRNA, it gives insights into localization of active bacteria within the mucosa. Their work highlighted the strong prevalence of *B. fragilis* within the mucosa of IBD patients and mostly CD patients (Swidsinski *et al.*, 2005). Our results are consistent with their observation as the AIR

of *B. fragilis* was the highest in CD patients (compared to healthy and UC individuals). The *B. fragilis* genome has been fully sequenced, analysed and led to the description of the phase inversion phenomenon in this species, with antigenic variation. The composition of the ten different polysaccharide biosynthesis gene clusters identified (seven with associated invertible promoters) suggested a mechanism of synthesis similar to the O-antigen capsules of *Escherichia coli* (Mazmanian *et al.*, 2005). Mazmanian *et al.* (2005) highlighted that *B. fragilis* expressing a specific surface polysaccharide (PSA presenting a zwitterionic structure) was sufficient to correct immunological defects observed in the absence of a commensal microbiota in axenic mice models. In the colonization phase, the *B. fragilis* PSA was able to mediate establishment of a proper T helper 1/T helper 2 cell balance through specific production of Th1 cytokines. More recently, the same group showed that *B. fragilis* protected its host from inflammatory disease caused by *Helicobacter hepaticus* in an experimental model of colitis and that this anti-inflammatory effect was mediated by the PSA molecule (Mazmanian *et al.*, 2008). However, the LPS of *B. fragilis* has been shown to trigger inflammatory events via the Toll-like receptor 2 and is likely to be involved in systemic inflammatory response syndrome caused by gut bacteria (Erridge *et al.*, 2004). However, this might not be paradoxical. Hence, Netea *et al.* (2004) observed that in patients with CD bearing the 3020insC mutation on *NOD2*, stimulation with *B. fragilis* led to a significantly lower interleukin 10/tumour necrosis factor alpha ratio than when wild-type *NOD2* or healthy volunteers' cells were stimulated.

Our results showed a clear discrepancy between intestinal diversity or richness measured as the presence of 16S rRNA gene signatures of bacteria and their transcriptional or metabolic status (rRNA). Transcriptional activity is also a prerequisite for intestinal colonization of bacteria and can help in differentiating active bacteria from bacteria that were merely present (Lorenz & Wackernagel, 1987; Josephson *et al.*, 1993; Wagner, 1994). It was noteworthy that the most active bacteria associated with the human gut mucosa were also the less abundant. Indeed, the highest AIRs were observed for bacteria representing less than 1% of total microbiota in each of the clinical groups. This phenomenon has also been observed at the mRNA level in another complex ecosystem (ocean surface water) using high throughput sequencing. While comparing bacterial cDNA to bacterial DNA, Frias-Lopez *et al.* (2008) observed that protein clusters with the highest cluster-based expression ratios also tended to fall into the low abundance category.

The role of the metabolic activity of specific bacteria or bacterial populations towards the pathogenesis of IBD remains unclear. A potential function is the production of health-promoting metabolites or precursors, as recently shown for *Roseburia* sp. (Devillard *et al.*, 2007). Differential activity of intestinal bacteria could also be the consequence of a complex self-regulation network via quorum-sensing

mechanisms, which will arise as an important field of research in the future (Dunny *et al.*, 2008).

Our study shows that investigation of the transcriptional activity of intestinal bacteria can provide important additional information for the understanding of the complex composition of the intestinal microbiota. Data on the functional status of intestinal bacteria will increase the degree of complexity of the intestinal network that has been determined so far and will help in the understanding of regulatory processes.

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