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METAGENOMIC APPROACHES TO UNRAVEL THE COMPOSITION AND FUNCTION OF THE HUMAN SMALL INTESTINE MICROBIOTA

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SUMMARY

The small intestine microbiota remains largely unexplored, which is a consequence of the poor accessibility of this ecosystem. Nevertheless, this part of the intestine is of great importance for physiological homeostasis of the host. Not only is the small intestine the major site of nutrient absorption, but it also provides the most important mucosal immune organ of our body. The interaction of the small intestine mucosa with the residing luminal and adhered microbial populations is bound to represent an important cross-talk repertoire that is importance for health. The review presented here, provides the insights obtained in small intestinal microbiota composition and function. The model system used for these studies is that of ileostoma-individuals that lack a colon but have a normally functioning small intestine. The studies encompass phylogenetic community composition analyses, as well as metagenome and metatranscriptome analyses. The results presented highlight several aspects of small intestine community structure and function that generate a clear and comprehensive view of this habitat and the selective forces that shape its residing microbial community.

INTRODUCTION

The human gastrointestinal (GI) tract is inhabited by a consortium of microorganisms that is strongly dominated by bacteria and is referred to as gastrointestinal microbiota (*Guarner*, 2006; *Leser* and *Molbak*, 2009). Besides bacteria the presence of Eukarya (*Scanlan* and *Marchesi*, 2008) and Archaea (*Dridi* et al., 2009; *Eckburg* et al., 2005) in the human GI tract has been reported, albeit with relative low abundance and diversity. Moreover, an impressive viral community has been detected in the human intestine using meta-analyses and revealing 1,200 viral genotypes in faeces obtained from adult subjects (*Breitbart* et al., 2003). Based on their dominance in the intestinal ecosystem, most attention has been given to the bacterial communities in this system. Traditional approaches have employed cultivation as the main method to study the microbial community in the human intestine. However, the application of molecular methodologies to unravel bacterial

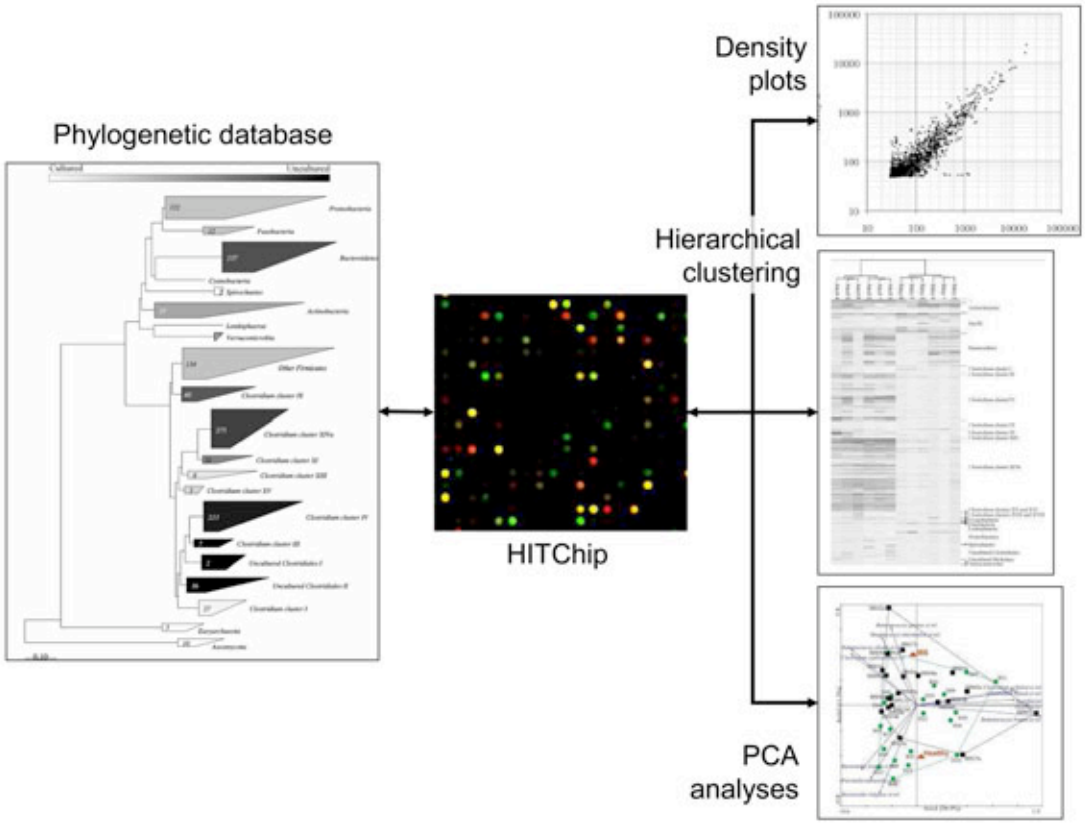

Figure 1: Schematic representation of the application of the Human Intestinal Tract Chip (HITChip) designed at Wageningen University (*Rajilic-Stojanovic* et al., 2009), which is based on the Agilent format slides, containing probes designed for the variable regions 1 and 6 (V1 and V6) of the 16 S rDNA sequences of more than 1200 non-redundant bacterial OTUs. Data acquired by HITChip provide direct phylogenetic connection to the underlying probe-phylogeny assignment database. A series of processing scripts enables effective and (semi-) quantitative interpretation of the array data in terms of microbiota composition at various levels of phylogenetic depth (i.e., from phylum down to species), which can be visualized using various graphical modules, and can be analyzed by a variety of statistical and/or clustering software packages that are linked to the array database.

community structure in the human intestine that has been applied since approximately 15 years revealed that cultivation approaches have severely underestimated the bacterial population diversity and underlined that the majority of the bacterial species residing in this habitat appear to be uncultured to date (*Zoetendal* et al., 2008).

The highly diverse bacterial community residing in the human GI tract (Figure 1) is dominated by phylotypes belonging to the Firmicutes, Bacteroidetes, and Actinobacteria (*Backhed* et al., 2005; *Eckburg* et al., 2005; *Rajilic-Stojanovic* et al., 2007). Although Firmicutes are found in the intestine of all mammals, each mammalian species harbours a distinct microbial composition showing relatively limited variation at intraspecies level (*Ley* et al., 2008a). Intestinal microbiota composi-

tions of different mam-mals can be clustered by dietary habits revealing distinct community structures in carnivores, omnivores and herbivores, which can be roughly characterized by increasing microbiota diversity, respectively. In addition, discriminative clustering could be achieved on basis of host phylogeny and cognate intestinal anatomy. Consequently, it is not surprising that the human intestinal microbiota composition resembles that of omnivorous primates (*Ley* et al., 2008a,b).

Recently the interest in understanding intestinal community structure and function has increased on basis of observations that indicate correlations between the human intestinal microbiota and human health and disease. This field may eventually provide microbiota-based diagnostic markers for health and disease, while such insight may also offer avenues towards diet or microbial intervention strategies to prevent or treat human diseases via modulations of the intestinal microbiota. However, the success rate of these latter possibilities will depend on the causal relations underlying the observed correlations between intestinal microbiota and health and disease.

MOLECULAR METHODS TO UNRAVEL BACTERIAL COMMUNITY STRUCTURE IN THE HUMAN INTESTINE

Culture independent molecular methods to determine bacterial community structure commonly target the universal bacterial phylogenetic marker, 16S ribosomal rRNA (rRNA) or its encoding gene. Frequently applied 16 S rRNA methods to assess (quantitative) composition and diversity of the microbiota on basis of 16 S rRNA include classical cloning and sequencing, DGGE/TGGE, FISH, and qPCR. These methods provide different degrees of sensitivity, selectivity and phylogenetic resolution and have been employed to determine bacterial-community structures, or detect and/or quantify specific bacterial groups within a variety of samples derived from the human intestine. Both these methodologies as well as their overall results have been reviewed recently (*Zoetendal* et al., 2004) and will not be discussed here. Nevertheless, two emerging technologies, i.e. phylogenetic microarray analysis and barcoded pyrosequencing, will be reviewed here since they offer novel, high-throughput in depth composition profiling possibilities.

Phylogenetic microarrays are commonly constructed by printing 16S rRNA-targeting oligonucleotide probes on a carrier surface (in many cases glass slides). This platform enables high-throughput, in depth, semi-quantitative characterization of microbial communities (*DeSantis* et al., 2007; *Paliy* et al., 2009; *Rajilic-Stojanovic* et al., 2009; Figure 1). An obvious constraint of phylogenetic microarrays is their restriction to detect phylogenetic groups that are represented in the array design (*Palmer* et al., 2006), which may provide incomplete composition impressions in specific samples (as an example see below). Therefore, array design should preferably be updated on a regular basis to incorporate newly identified bacterial groups that inhabit the target niche, e.g. the human colon. An alternative methodology that holds great promises for high-throughput analysis of microbial composition in human intestinal samples (or any other environmental sample) is designated barcoded 454 pyrosequencing (*Andersson* et al., 2008). This method is based

on sequencing-based *de novo* community profiling, and consequently is not restricted to 16S rRNA sequences that are known beforehand. This technology is compatible with high-throughput analyses and provides relatively high phylogenetic resolution. Pyrosequencing has been employed to unravel the microbial community structure in various samples obtained from humans, including the intestinal tract (for a review see: *van den Bogert* et al., 2010). Importantly, deep pyrosequencing and phylogenetic microarray analysis of the microbial community of faecal samples generated comparable results (*Claesson* et al., 2009; *van den Bogert* et al., 2010). Pyrosequencing-data interpretation is not trivial due to the huge amounts of sequences generated and requires stringent sequence quality control and effective taxonomic profiling, interpretation and visualization software suites. The level of sensitivity of the pyrosequencing method depends on the amount of the sequences generated for an individual sample, and probably requires a 10- to 100- fold excess of the depth that is targeted for.

Pyrosequencing, like other PCR-based profiling technologies suffers from the potential biases introduced by the amplification-primers used as well as by intrinsic biases of the DNA amplification reaction itself. Due to the continuous expansion of the 16S rRNA database, primers (and probes for application in FISH) tend to become outdated and therefore require constant updating to ensure appropriate coverage of the targeted microbial population (*Baker* et al., 2003). The PCR amplification of highly conserved genes like the 16S rRNA gene intrinsically suffers the risk of chimera formation, which may contaminate the databases with biologically irrelevant sequences that are falsely assigned to specific bacterial groups (*Ashelford* et al., 2005). Recently the field of random shot-gun sequencing of environmental DNA (metagenomics; *Handelsman,* 2004) has expanded drastically as a consequence of the development of extreme-throughput sequencing technologies (454-Titanium; Illumina and Solid), which will enable the development of alternative methods for community composition profiling that are based on function pattern determinations rather than the single marker 16 S rRNA gene. A landmark achievement in this field is the recent release of a human intestine microbiota reference gene-set that contains more than 3 million bacterial genes discovered within the intestinal community. This large amount of genetic information of the human intestine microbiota offers an unprecedented level of resolution for function based profiling for ecosystem communities (*Qin* et al., 2010). For example this database can be employed as a reference template for profiling and pattern recognition using high-throughput short-sequence DNA information as can be obtained from next-generation sequencing technologies like Illumina or Solid.

THE LARGE AND SMALL INTESTINE OF HUMANS

The bacterial community is not evenly distributed over the different regions of the human gut; it increases in density along the longitudinal axes. The bacterial populations of the stomach are relatively small (10^3-10^4 bacteria per gram of contents), which is due to the harsh conditions encountered in this habitat such as very low pH values (approximately 2.5 in humans) and

other antimicrobial factors (*Guarner*, 2006; *Leser* and *Molbak*, 2009). The diversity of stomach microbiota is low, and merely 128 phylotypes (or operational taxonomic unit: OTU) could be recovered from 23 individuals (*Bik* et al., 2006).

Once entering the small intestine, bacterial densities increase to approximately 10^4 to 10^5 bacteria in the jejunum and 10^8 or even more bacteria in the terminal ileum. Climax community densities are reached in the large intestine where more than 10^{11} bacteria per gram contents have been reported (*Guarner*, 2006; *Leser* and *Molbak*, 2009). Based on their accessibility, faecal samples are often used to study the microbiota in the large intestine (*Huys* et al., 2008). However, several studies have shown the marked difference between bacterial community structures in faecal samples as compared to those adhered to the colonic mucosa (*Eckburg* et al., 2005; *Lepage* et al., 2005; *Zoetendal* et al., 2002). The large intestine microbial community is highly complex and has been estimated to encompass a complexity that spans at least 500 phylotypes (*Eckburg* et al., 2005), which predominantly classify within the phyla Firmicutes, Bacteroidetes, and Actinobacteria. The large intestine microbiota composition appears to be very individual-specific (*Zoetendal* et al., 2008) and displays considerable stability over time and resilience following antimicrobial interventions like antibiotic treatments etc. (*Matsuki* et al., 2004; *Rajilic-Stojanovic* et al., 2009; *Zoetendal* et al., 1998). Nevertheless, a recent study identified a potential group of potential core microbial phylotypes that inhabit the majority of humans at high relevant abundance (*Tap* et al., 2009). Analogously, faecal microbiota profiling by phylogenetic microarrays, revealed a set of responding probes that were shared among the individuals (*Rajilic-Stojanovic* et al., 2009). Inversely, the vast majority (~80%) of the detected phylotypes appears to be host specific (*Tap* et al., 2009). Despite the observed composition variation among the intestinal microbiota in human individuals, metagenomic analysis has recently indicated that there appears to be a remarkable functional congruency in these different microbial communities (*Turnbaugh* et al., 2009). Analogously, abundance profiling of the reference gene set of the human microbiome (*Qin* et al., 2010) in individuals reveals that the majority of genetic functions is conserved among the faecal microbiota of individuals, while a portion (~10% of the complete 3.3 million genes identified) of specific genes is actually shared among all individuals and can be regarded as a core metagenome. Moreover, gene frequency analysis comparing human intestine metagenome datasets and whole bacterial genomes or metagenome data sets obtained from other environmental niches led to identification of gene sets that are specifically enriched within the human faecal metagenome (*Qin* et al., 2010).

Recent research has exemplified that a healthy human host and its intestinal microbiota coexist in a homeostatic relationship (*Hooper*, 2009; *Leser* and *Molbak*, 2009; *Macpherson* and *Harris*, 2004). The intestinal microbiota benefits from a stable environment and nutrient supply that are provided in the intestinal tract, while the host gains products from microbial fermentation conversion of host indigestible components into short chain fatty acids (SCFA; acetate, propionate, butyrate; 10% of our energy requirement), vitamin K and B12 production and protection against potential pathogens (*Guarner*, 2006; *Leser* and *Molbak*, 2009; *Macpherson* and *Harris*, 2004;

Neish, 2009). Overall, the intestinal microbiota composition and activity patterns may have a pronounced effect on human health and several studies indicate that certain health disorders are associated with deviations in aberrations in the intestinal microbiota composition and/or function (for a recent review see: *Leser* and *Molbak*, 2009). Extending our knowledge of this microbial ecosystem therefore holds great promise for future interventions that aim to prevent or treat certain diseases or disorders though modulation of the intestinal microbiota community.

MODELS TO STUDY THE SMALL INTESTINE MICROBIOTA

Contrary to the large intestine, our knowledge of the microbiota that inhabits the human small intestine is limited. This is largely due to the sampling difficulties for this region of the GI tract that is notoriously difficult to access. Consequently, the small intestinal microbiota studies to date depended largely on biopsy specimens obtained during (emergency) surgery (*Ahmed* et al., 2007) or samples collected from sudden death victims at autopsy (*Hayashi* et al., 2005). Microbial analysis of biopsies from the jejunum and the distal ileum revealed a relatively low bacterial diversity in the jejunum mucosa with a microbial community dominated by *Streptococcus* sp. while a predominance of *Bacteroidetes* and *Clostridium* clusters IV and XIVa (according to the phylogeny proposed in *Collins* et al., 1994) was identified for the distal ileum (*Wang* et al., 2005). However, as a consequence of the relatively extensive procedures required for obtaining these samples, they may not represent the true small intestinal microbiota of a healthy individual and do not provide insights into population dynamics (*Booijink* et al., 2007).

One alternative to obtain small intestinal samples, which circumvents the sampling difficulties associated with the small intestine, makes use of individuals that underwent surgical removal of the colon due to cancer or inflammatory bowel disease (IBD) and as a result have the terminal ileum connected to a stoma. This ileostoma provides a unique opportunity to non-invasively and repetitively sample the contents of the terminal ileum (*Booijink* et al., 2007, 2010). A recent study indicated that the microbiota in the effluent samples from these ileostomy subjects does not represent that of the terminal ileum in healthy subjects due to the penetration of oxygen (*Hartman* et al., 2009). Although this study seems to contradict with recent findings by Booijink that showed high abundance of strict anaerobes in ileostomy effluents (*Booijink* et al., 2010), preliminary investigations in our laboratory with an orally introduced catheter revealed microbial communities were enriched in *Streptococcus* and *Veillonella* (belonging to *Bacillus* and *Clostridium* cluster IX, respectively) in jejunal and proximal ileal regions of the small intestine, while abundance of *Bacteroidetes* and *Clostridium* cluster XIVa were dominating in the terminal ileum (Figure 2), resembling the microbiota in ileostoma effluent and the colon, respectively. These results suggest that ileostoma effluent is probably not an appropriate reflection of the terminal ileum lumen, but more proximal regions of the small intestine. Since these regions are among the first to interact with dietary components, it seems that the ileostoma model system provides an excellent model to study these early

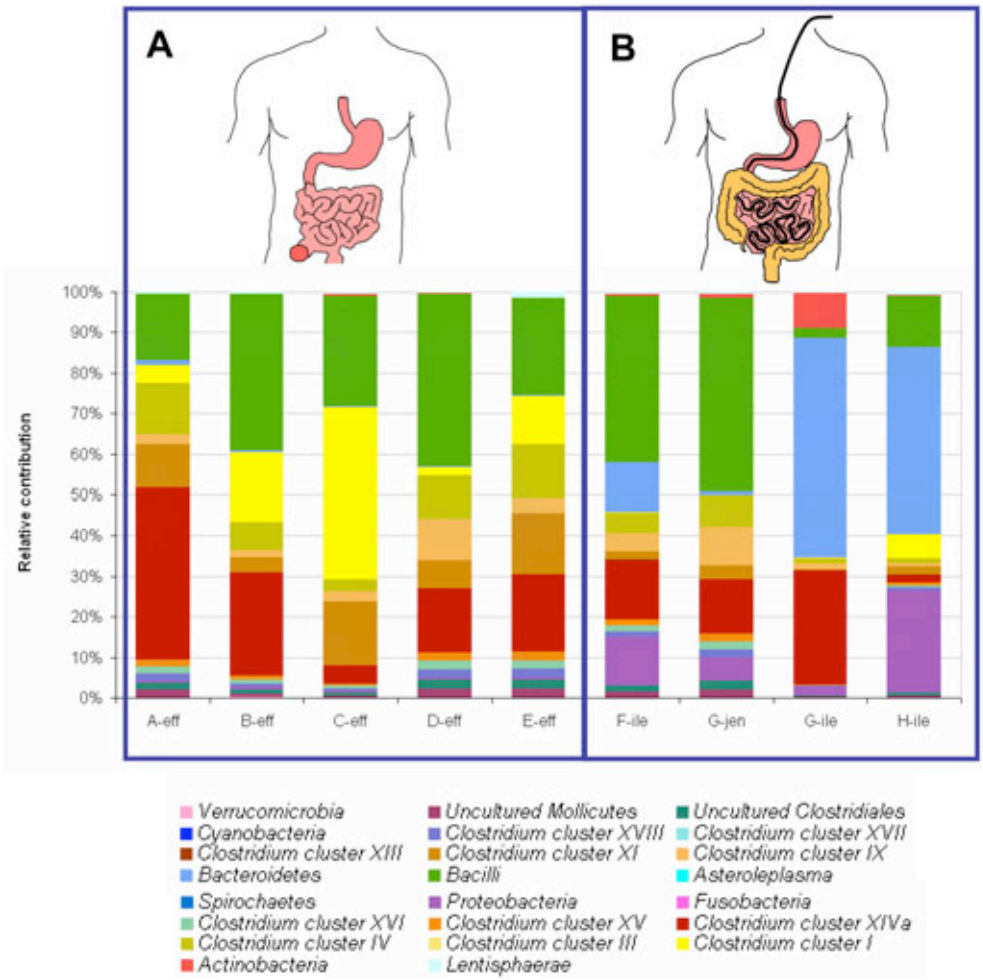

Figure 2: Schematic representation of the two small intestinal models employed, i.e., the ileostoma model (A) and the extended oral catheter sampling (B). The relative contributions of detected phylogenetic groups are shown for ileostoma effluent of 5 ileostoma individuals (A-F), and catheter obtained small intestinal samples of 3 healthy individuals (F, G, and H) from the indicated regions of their intestine (ileum; ile, and jejunum; jen) (adapted from *van den Bogert* et al., 2010).

microbiota-diet-host interactions (Figure 2). The terminal ileum of normal individuals appears to resemble a mixed population of jejunal and proximal-ileal communities and those from the colon, which may be maintained through colonic reflux into the terminal ileum.

SMALL INTESTINAL MICROBIOTA AND ITS INTERACTIONS WITH DIET AND HOST MUCOSA

The small intestinal microbiota is the first microbial community of the intestine that interacts directly with the diet. Consequently, the small intestine microbiota has to compete with the absorptive capacity host mucosa for the

available carbon and nitrogen sources. Moreover, the physico-chemical conditions of the small intestine are harsh from a microbe's point of view, e.g., residence times are short and exposure to bile acids and host-digestive enzymes is constant. Therefore, it can be anticipated that the small intestine microbiota community is strongly influenced by changes in dietary composition that address microbial capacities differentially (*Booijink* et al., 2010). These dietary interactions can be expected to be more direct and more pronounced as compared to the large intestine, where most readily utilizable components of the diet have already been removed by absorption by the host and by the small intestine microbes. As a consequence the large intestinal microbiota focuses on the materials that have escaped the digestive capacities of the host and the small intestinal microbiota, and is commonly regarded as a large fermentative organ.

The small intestine harbours a large proportion of the body's immune cells, and thereby plays a prominent role in development and maintenance of appropriate immune homeostasis in newborn and adult mammals, respectively (*Brandtzaeg*, 1998; *MacPherson* and *Harris*, 2004). The intestine's mucosal tissue provides a highly sophisticated barrier that prevents inflammation of the underlying tissues by a complexly controlled and highly flexible innate and adaptive mucosal immune system. In this system, there is a particularly important role in induction and regulation of mucosal immunity for the immune-dedicated GALT (gut-associated lymphoid tissue) system, including important roles for Peyer's patches and mesenteric lymph nodes. The density of these dedicated immune sensing and modulation systems is much higher in the small intestine as compared to the large intestine, indicating that the small intestinal microbiota can be considered a prominent driver of the host-immune system.

It seems plausible that the development of functional foods that aim to modulate the host's immune system are more likely to act in the small intestine, and may include the interaction with the endogenous microbiota in this region of the intestine. Based on the above, it is of utmost importance to improve our understanding of the small intestine microbiota composition and function in relation to diet, and how diet-associated changes of this community may affect the overall functioning of the host's immune system.

SMALL INTESTINE METAGENOMICS

To obtain insight into the genetic potential within the small intestinal microbiota, in our laboratory we have constructed a large-insert (fosmid cloning vector) metagenomic library from ileostoma effluent obtained from a healthy individual who has had the stoma for more than 20 years and does not need medication for stoma-related problems. Since previous work had established that the ileostoma effluent microbiota fluctuates over time (*Booijink* et al., 2010), the metagenome libraries were constructed from four different samples of this one individual to encompass as much of the overall diversity of its microbial community as possible. The overall fosmid metagenome library constructed encompassed 25,344 clones that on average contain approximately 25-30 kb of insert DNA, indicating that the overall library contains more than 700 Mb of genetic material from the microbes within this

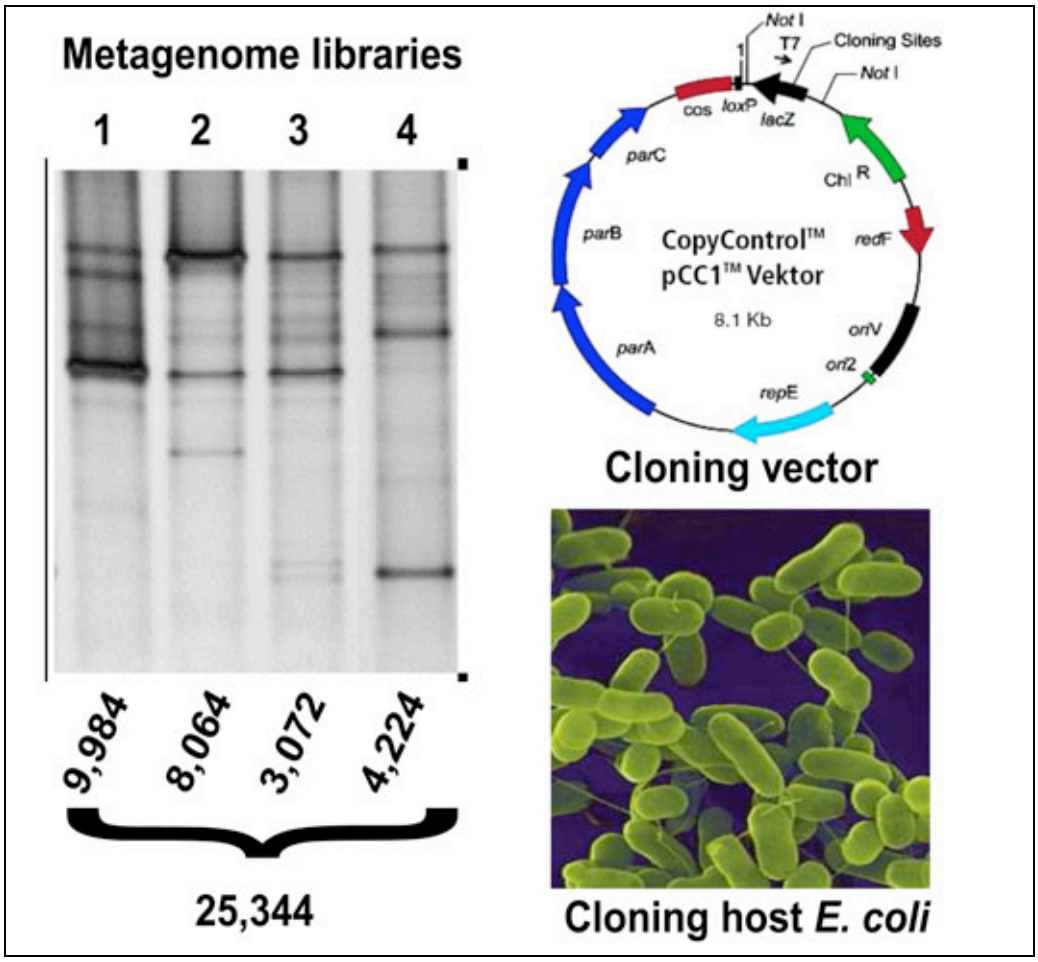

Figure 3: Schematic representation of the ileostoma effluent metagenome library construction, highlighting the diversity variation of the 4 samples used by DGGE microbiota profiling (left panel), and the numbers of fosmid clones obtained per sample. The right hand-panel shows the genetic map of the fosmid cloning vector used and a microscopic image of the *Escherichia coli* cloning host.

niche (Figure 3). Although library constructions are not required anymore for a sequence-driven metagenomics approach due to the development of cloning-independent next-generation sequencing technologies, the main reason for construction of this large-insert metagenome library was to enable the linkage between insert-sequence and functional properties per clone as they can be obtained through function-based high-throughput screening (*Handelsman*, 2004).

Various sequencing efforts were employed to investigate the diversity within the fosmid library inserts, including end-sequencing of all clones, and random sequencing of all libraries by 454-Titanium sequencing. Overall the entire sequence analyses performed generated approximately 178 Mbp of raw-sequence information. Phylogenetic positioning of all sequence reads indicated that the sequences originated from a wide variety of phylotypes, and *Clostridium* sp. *Streptococcus* sp., as

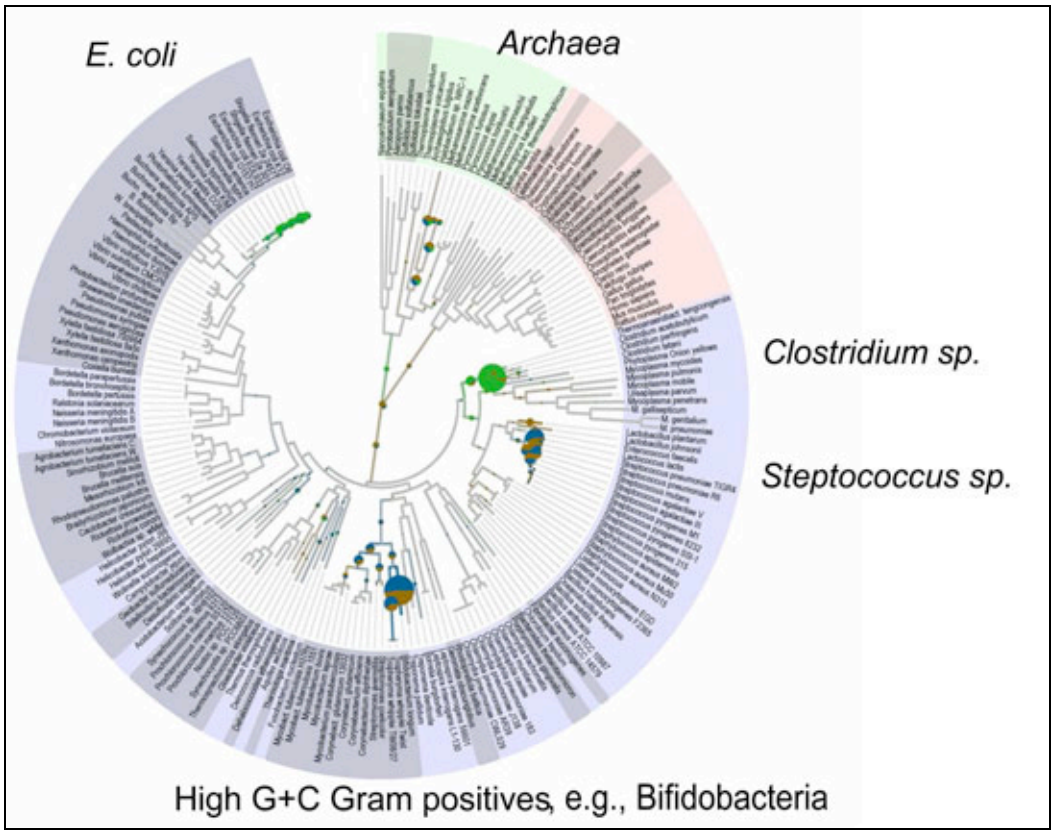

Figure 4: Phylogenetic positioning of the sequences obtained for 3 of the 4 metagenome libraries (differentially colour-coded) of the small intestine microbiota.

well as coliforms were detected among the dominant phylogenetic groups (Figure 4). Comparison of the phylogenetic profiling of the sublibraries 1, 2, and 3 revealed that all groups found in the overall analysis were found in each of the sublibraries. However, group distribution was not equal in the different sublibraries (Figure 4). The phylogenetic distribution of samples taken on the same day (2, and 3) displayed the highest similarity, and was separated from a sample that was taken a long time earlier (i.e., 1 year earlier, sample 1). The *Streptococcus* sp. and high G+C Gram-positives were overrepresented in the samples 2 and 3, while *Clostridium* sp. and coliforms were overrepresented in sample 1. This finding confirms previous observations that indicate that the microbial community in ileostoma effluent is fluctuating in time, which contrasts to the rather stable composition in the colon (*Booijink* et al., 2010). In addition, it underlines that metagenome analyses that target a single time point (i.e., the number of samples generally analyzed for intestinal metagenomics) may underestimate the overall genomic complexity encompassed within a niche, and eliminates the possibility to address the metagenome in the light of population dynamics.

Assembly of the reads per sublibrary revealed that 146 Mb of the overall sequence information could be assembled into contigs that in total en-

compass 63 Mb, leaving only 13% of the reads unassembled. This proportion of sequence reads that could be assembled is significantly higher than previously observed with faecal metagenomes (*Gill* et al., 2006; *Kurokawa* et al., 2007), containing 78 Mb and 727 Mb, respectively. This observation clearly confirms that the microbial community in the small intestine is less diverse in composition compared to that of the colon. More than 170,000 genes could be assigned to the assembled small intestine metagenome. Functional annotation of all predicted genes revealed a relatively large fraction of (conserved) hypothetical genes of unknown function (>55%). The function distribution over functional categories of all genes that could be assigned to such a category appeared to resemble the function category distribution normally observed with whole genome sequencing of individual bacteria, indicating that the cloning strategy had not introduced a too severe selective over- or under-representation of specific functional categories.

Comparative metagenomics of small and large intestine metagenome databases indicated that several pathways and functions related to carbohydrate uptake and metabolism were highly enriched in the small intestine microbiota. In contrast, membrane proteins, enzymes related to metal binding and proteins with unknown functions were enriched in the faecal microbiota. KEGG module mapping of small intestine-enriched functions underpinned a strong overrepresentation of sugar transport systems (especially PTS), and functions associated with the central carbon and energy metabolism (e.g., glycolysis and pentose phosphate pathway). For the latter category, the pathways involved in biosynthesis of sulphur containing amino acids. Similar enrichments were observed in differential analyses of categories of orthologous genes (COG) classes. The phylogenetic distribution of the genes related to central metabolic pathways was not restricted to a single phylum, but appeared to scatter across a variety of both Gram-positive and Gram-negative organisms, with slight overrepresentation of the Streptococci. This indicates that the small intestine microbiota harbours an extensive repertoire of rapid-sugar-import functions and the corresponding pathways involved in their utilization to support energy generation and growth. Next to the enrichment of these glycobiology-associated functions, a strong enrichment of the biosynthetic pathways leading to co-factor such as the vitamins cobalamin and biotin were observed. This finding may be relevant for host physiology, since biotin absorption by epithelia is known to take place in the distal region of the small intestine (*Said*, 2009), suggesting that microbes in the small intestine may significantly contribute to the human biotin supply.

Since the metagenome provides only insight into the genetic potential of the ecosystem, the actual activity pattern of the microbial community was investigated by metatranscriptomic analysis using total RNA obtained from ileostoma effluent samples. Since total bacterial RNA contains 95-99% of rRNA the sample was enriched for mRNA by selective capture methodology (Ambion microbe-Express) prior to analysis by cDNA construction and cloning. Sequencing cDNA libraries revealed that despite this enrichment still approximately 50% of all sequences were derived from rRNA, indicating that this step could be further improved. The function pattern observed in the metatranscriptome appeared to be enriched for PTS and other carbohydrate transport systems as well as glycolytic and pentose phos-

phate pathways in comparison to the metagenome of the same samples, further strengthening the importance of these functional categories in this niche. In addition, various fermentation pathways appeared to be highly expressed in the small intestine microbiota with an emphasis on those leading to production of lactate, proprionate and acetate. Taken together these findings indicate that rapid fermentation of carbohydrates is likely an important microbial characteristic that enables individual species to successfully colonize the harsh habitat of the small intestine (*Zoetendal* et al., 2010). Phylogenetic profiling of these mRNA derived sequences indicated that the majority of the transcripts detected derived from *Streptococcus* sp. and coliforms. In comparison to the metagenome database especially the fast growing facultative anaerobic organisms appeared to be prominently represented at the transcription level, suggesting high levels of gene activity in the microbes belonging to this group.

Among the dominant groups of bacteria in the small intestine that displayed the highest activity are the streptococci, which are renowned fast growing facultative anaerobic bacteria that can rapidly import and ferment relatively simple carbohydrates like mono- and disaccharides. To successfully utilize such carbohydrates in the small intestine these microbes have to compete with the host for many of these substrates. Consequently, the PTS and other transport systems as well as the downstream conversion pathways (e.g., glycolysis, pentose phosphate pathway) need to be efficient and highly expressed, which was clearly reflected by the metatranscriptome sequences. Although PTS systems of *E. coli* and relatives were also found in the metatranscriptome, their expression and the *E. coli* abundance were less compared to those of *Streptococcus* sp., suggesting that the latter bacteria most effectively fulfil the selective demands of the small intestinal habitat.

Our current model that aims to represent the small intestine microbiota focuses on the capacity for fast adaptation to the fluctuating conditions and variable nutrients as a predominant determinant for successful colonization of the small intestine habitat.

FUTURE PERSPECTIVES

This small intestine is of great importance for development and maintenance of immune homeostasis in humans. Therefore, the residing microbes may play a prominent role in tuning/modulating the local mucosal immune system, which is likely to also impact on systemic immune homeostasis. The studies presented above shed first light on the microbiota community of the human small intestine. Further studies of this dynamic microbial ecosystem are of great importance to understand its relation with host immunity. In addition, it is highly likely that dietary interventions are very effective for the modulation of the small intestine microbial community composition and function. The relative simplicity of the microbial community in the small intestine and the emerging evolutionary drivers that shape this community, enable a designer approach towards functional foods aiming to change small intestine microbiota composition and function with the ambition to modulate host immune system homeostasis. An example of such interventions may be found in the consumption of probiotics, which can drastically alter the microbial community in the small intestine temporarily and can thereby affect the

mucosal biology and immune system. Illustrative for such possibilities is the recent publication that shows that oral administration of a model probiotic organism, i.e. *Lactobacillus plantarum*, can alter mucosal gene expression patterns associated with immune regulatory networks (*van Baarlen* et al., 2009). An alternative to probiotics may lie in the composition of specific food products that stimulate/repress specific sub-groups of the endogenous small intestine microbiota aiming to alter the luminal antigen repertoire and thereby modulating local mucosal immunity. In view of the above, interventions that include administration of, or elimination of specific glyco-compounds from the diet could stimulate specific subpopulations (and/or repress other subpopulations) of the small intestine microbiota, which may be a highly effective approach to altering such luminal antigen repertoires.

Approaches towards such functional food studies that include microbiota and small-intestine mucosal analysis may employ the ileostoma subjects as a model system, since these individuals provide easy, non-invasive access to sequential samples from the small intestine of a single individual without requirements for invasive methodologies. Such studies can provide leads to dietary manipulation of the small intestine and systemic immune function by targeted alterations of microbial communities that interact with the small intestinal mucosa.

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