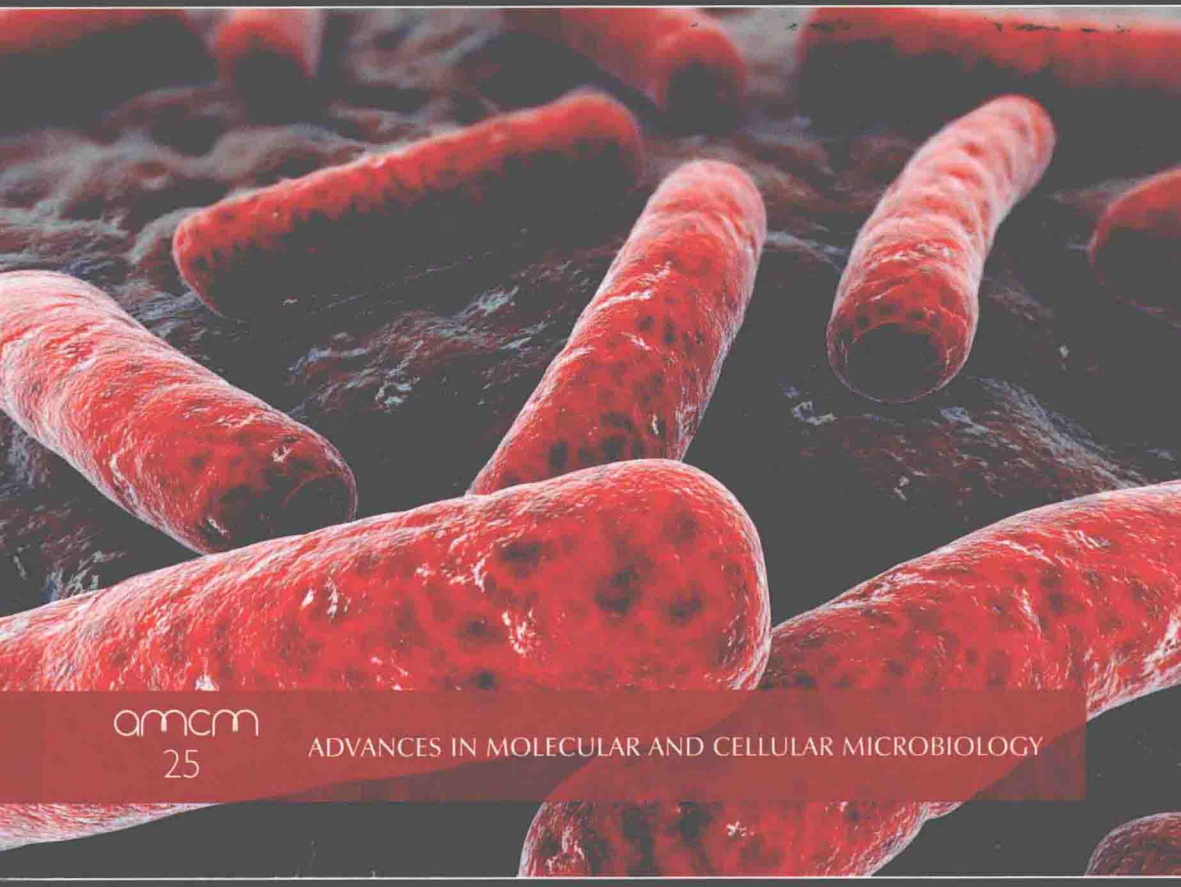
The Human Microbiota and Microbiome

Edited by Julian R. Marchesi



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Julian R. Marchesi

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Advances in Molecular and Cellular Microbiology

Through the application of molecular and cellular microbiology, we now recognize the diversity and dominance of microbial life forms on our planet, which exist in all environments. These microbes have many important planetary roles, but for us humans a major problem is their ability to colonize our tissues and cause disease. The same techniques of molecular and cellular microbiology have been applied to the problems of human and animal infection during the past two decades and have proved to be immensely powerful tools in elucidating how microorganisms cause human pathology. This series has the aim of providing information on the advances that have been made in the application of molecular and cellular microbiology to specific organisms and the diseases that they cause. The series is edited by researchers active in the application of molecular and cellular microbiology to human disease states. Each volume focuses on a particular aspect of infectious disease and will enable graduate students and researchers to keep up with the rapidly diversifying literature in current microbiological research.

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Contents

1 The Stomach and Small and Large Intestinal Microbiomes

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1.1 Introduction

This introductory chapter provides an updated overview on the composition of the microbiome in the human gastrointestinal tract (GIT); that is, the microbiota of the GIT together with its entire genetic information and the microbe–microbe and host–microbe interactions taking place in this habitat. More specifically, recent scientific advances on the microbiome of the upper (stomach and duodenum) and lower GIT (jejunum, ileum, caecum, colon, rectum), particularly of healthy adults, will be discussed. However, where necessary, some studies performed with diseased patients or animal models will also be presented and integrated into the state-of-the-art-knowledge about the human GIT microbiome. In addition, an update on factors shaping the composition of the GIT microbiome will be given. For a more functional or physiological discussion of the human intestinal microbiome, the reader is referred to Chapter 6, this volume. The structure and function of the microbiome of the uppermost part of the human digestive system, i.e. the oral cavity, are presented and discussed in Chapter 2 of this volume.

From a microbiological point of view, the human GIT can be regarded as the best investigated ecological niche of the human body, although some difficulties exist in obtaining representative samples from various parts of the GIT. Moreover, the human GIT probably represents one of the best investigated microbial ecosystems on earth. This fact can be explained due to the great importance of the GIT microbiota in maintaining and driving human health, disease and well-being: on a quantitative basis, humans can be regarded as a super-organism, consisting of 90% microbial cells and even 99% microbial genes, and the vast majority of the microbial diversity is located in the human GIT (Wilson, 2008). Consequently, the general importance of the GIT microbiome for human health and disease regarding digestion and general metabolism, gut development or immune status is undoubted. Hence, a wealth of literature on the human GIT micobiome is already available, including several current and comprehensive review articles and reviewing book chapters (Wilson, 2008; Doré and Corthier, 2010; Marchesi, 2010; Gerritsen *et al.*, 2011; Walter and Ley, 2011; Willing and Jansson, 2011). For a complementary overview including some of the more classical literature about the human GIT microbiome, the reader is referred to these articles.

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 The Human Microbiota and Microbiome (ed. J.R. Marchesi)

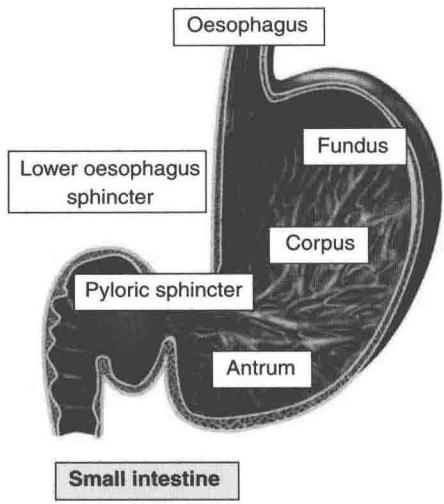
1.2 The Microbiota of the Human Stomach

1.2.1 Environmental conditions

The human stomach (Fig. 1.1) is a J-shaped structure with a volume of approximately 1.5 l. It can be differentiated into an upper part (fundus), the main body (corpus) and a lower part (antrum), which is connected to the duodenum part of the small intestine via the pyloric sphincter. The folded stomach epithelium is covered by a protective mucus layer of up to 600 μm thickness. The main functions of the human stomach are temporary food storage, mixture of food and gastric juice to chyme, pre-digestion of proteins by acidic pH and pepsin, and disinfection of the ingested food. The environmental conditions in the stomach are eutrophic – due to ingested food, mucus, desquamated epithelial cells and dead microbes – aerobic and acidic, with a more or less constant temperature of 37°C, i.e. the body temperature of the host. Pronounced daily fluctuations in temperature, pH (from pH 1 to pH 5) and available nutrients are common and linked to ingestions of food and beverages. Bacterial viable counts are strongly dependent on the actual gastric pH and range from 10^3 to 10^6/ml (Wilson, 2008; Walter and Ley, 2011).

1.2.2 Composition of the stomach microbiota

Data on the human stomach microbiome are usually collected by investigating biopsies, taken endoscopically after several of hours of fasting. Despite the harsh and antimicrobial environment, recent molecular diversity studies – in particular the widely cited study by Bik and co-workers – have shown, surprisingly, that the human stomach contains a diverse, unevenly distributed microbial community dominated by *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Actinobacteria* (Bik *et al.*, 2006). In endoscopic biopsies taken from 23 North American patients with symptomatic upper gastrointestinal disease, they identified 128 phylotypes from 8 phyla by a 16S rRNA gene clone library approach. Several more recent studies corroborated that a remarkable diversity of bacterial genes could be amplified and identified from the human stomach (Andersson *et al.*, 2008; Dicksved *et al.*, 2009; Li *et al.*, 2009;



Current knowledge

- remarkable undisputed bacterial diversity: 7–13 phyla, >> 100 phylotypes
- presence of *Helicobacter* sp. dramatically reduces bacterial diversity
- key genera: *Helicobacter*, *Streptococcus*, *Prevotella*

Key questions

- differentiation of truly resident from transient, i.e. food-, mouth- or oesophagus-derived species
- functional relevance of the resident microbiota (other than *Helicobacter* sp.)
- effect of presence/absence of *Helicobacter* sp., diet, ethnicity and gastric diseases (cancer) on community composition

Fig. 1.1. Current knowledge and key questions regarding the microbial ecology of the human stomach.

Maldonado-Contreras *et al.*, 2011). While investigating ten *Helicobacter pylori*-free patients with a Chinese background, Li and co-workers quite clearly corroborated several key findings of the American-based study of Bik and colleagues (Li *et al.*, 2009). With respect to the total number of detected phylotypes (133 versus 127), the number of phyla (8 versus 7) and the most abundant two genera (*Streptococcus* and *Prevotella*), both studies yielded strikingly similar results. Anderson and co-workers even detected 262 phylotypes representing 13 phyla in biopsies of the stomach of three *H. pylori* negative patients with peptic ulcers (Andersson *et al.*, 2008). As a consequence, the human stomach can no longer be considered a mono-associated environment.

In the thick mucous layer overlying the gastric epithelium, non-acidophilic bacteria can also be found, in particular *H. pylori*. When present, *H. pylori* usually dominates the stomach bacterial community (Andersson *et al.*, 2008). So far, *H. pylori* is the only bacterium of the human stomach that can be considered unambiguously as a true resident and is considered to contribute to the development of gastritis, peptic ulcers and even gastric cancer (Dorer *et al.*, 2009).

Several recent studies have tried to unravel correlations between the composition of the microbial community in the stomach and the *H. pylori* status of patients. In a study by Maldonado-Contreras and colleagues, which was focused on patients from developing countries, a positive *H. pylori* status was correlated with increased relative abundances of (non-*Helicobacter*) *Proteobacteria*, *Spirochetes* and *Acidobacteria*, while *Actinobacteria*, *Bacteroidetes* and *Firmicutes* were less abundant (Maldonado-Contreras *et al.*, 2011). However, the study also showed that ethnicity had a stronger impact on the stomach community composition than the *H. plyori* status. Focusing on *H. pylori* negative patients, Li *et al.* detected significantly higher abundances of *Firmicutes*, in particular *Streptococcus* spp., in the stomach mucosa of patients with antral gastritis (Li *et al.*, 2009). Interestingly, *Streptococcus* spp., together with bacteria of the genera *Lactobacillus*, *Veillonella* and *Prevotella*, were also abundant members of the stomach community in a study on patients with gastric cancer and a low *H. pylori* abundance (Dicksved *et al.*, 2009). However, no statistically significant differences were found between the stomach community of cancer and non-cancer patients.

1.2.3 Resident or transient microbiota?

Approximately 10^{10} microorganisms enter the human stomach every day. As a consequence, a clear differentiation of truly resident from just transient (swallowed) microbial species is difficult. Indeed, the majority of the 33 phylotypes identified in the stomach of all three patients investigated by Andersson *et al.* were affiliated with the genera *Streptococcus*, *Actinomyces*, *Prevotella* and *Gemella*, which were also abundant in the throat community (Andersson *et al.*, 2008). However, streptococci were shown to survive in the stomach and to adhere tightly to the mucosa, suggesting they might truly represent resident stomach species (Li *et al.*, 2009). Acid tolerance is clearly a prerequisite for (even just transient) microbial survival in the stomach lumen, and this is why particularly acid-tolerant streptococci, lactobacilli, staphylococci and *Neisseria* spp. have frequently been found in the stomach lumen. It was suggested that some of these bacteria be investigated in more detail for potentially beneficial (probiotic) properties (Ryan *et al.*, 2008). A similar suggestion was recently also put forward for propionibacteria: Delgado and co-workers cultured propionibacteria – mostly affiliated with *P. acnes*, but devoid of any clear pathogenic properties – from gastric mucosa samples of 8 out of 12 healthy patients and proposed them as true residents of the human stomach (Delgado *et al.*, 2011).

So far, the functional relevance of the surprisingly high microbial diversity in the human stomach is still largely obscure (Lawson and Coyle, 2010). Its elucidation will require more long-term, dynamics-orientated and comparative analyses of mouth, throat and stomach communities and linking of particular physiological conditions, for example those associated with certain gastric

diseases and/or the presence/absence of *H. pylori*, with the composition of the microbiota of the stomach. Eventually, such studies might prepare a basis for the definition of novel therapeutic targets (Lawson and Coyle, 2010).

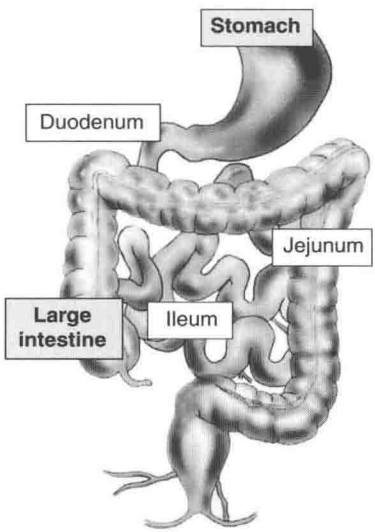
1.3 The Microbiota of the Small Intestine

1.3.1 Environmental conditions

In the small intestine, the vast majority of food components are digested by mostly host-derived hydrolytic enzymes and subsequently absorbed by the intestinal mucosa. The small intestine can be divided into three major parts (Fig. 1.2), with a more or less constant diameter (~3 cm) but considerable differences in length: i.e. duodenum (~25 cm), jejunum (~1.0 m) and ileum (~2.0 m). The entire epithelium of the small intestine is covered with a thick (up to 250 μm) protective mucus layer, secreted by goblet cells. In order to facilitate digestion and absorption, the surface area of the small intestine is greatly increased to almost 300 m^2 by the formation of villi and microvilli ('brush border'). On transfer through the pyloric sphincter, chyme from the stomach is mixed with intestinal juice (combined excretion of epithelial cells), pancreatic juice and bile by peristaltic movements. Compared to the large intestine, microbial growth is hampered in the small intestine by relatively short food retention times, antimicrobial peptides secreted by paneth cells and bile salts. However, growth conditions for microorganisms improve towards the end of the small intestine. Consequently, the numbers of luminal microorganisms increase from approximately 10^2 ml^{-1} in the jejunum up to 10^8 ml^{-1} in the terminal ileum (Wilson, 2008; Walter and Ley, 2011).

1.3.2 Composition of the small intestinal microbiota

Due to its restricted accessibility, the microbiota of the human stomach, and particularly of the small intestine, has been investigated much less intensively than that of the mouth and large intestine or faeces. In particular, data on the small intestinal microbiota of



Current knowledge

- dominance of facultative and obligate anaerobes (*Streptococcus* sp., enterobacteria, *Clostridium* sp., *Bacteroidetes*)
- increasing cell numbers, microbial diversity and share of anaerobes from duodenum towards ileum
- significantly lower diversity but higher temporal variability of microbial community compared to colon
- competition for carbohydrates with host

Key questions

- functional relevance of the resident microbiota for the host
- suitability of stoma patients as models due to potential influx of oxygen
- development of appropriate sampling techniques

Fig. 1.2. Current knowledge and key questions regarding the microbial ecology of the human small intestine.

healthy individuals are scarce. Until a few years ago, it was common knowledge that the lumen and mucosa of duodenum and jejunum were colonized at low density by only a few microorganisms, including acid-tolerant streptococci and lactobacilli. Towards the end of the ileum, the lumen was described as being dominated by streptococci, enterococci and coliforms, while in the mucosa, obligate anaerobes (*Bacteroides* spp., *Clostridium* spp., *Bifidobacterium* spp.) could also be found (Wilson, 2008, and studies cited therein). This knowledge has been broadened during the past few years.

In order to characterize the small intestinal microbiota in more detail by molecular means, Booijink and co-workers investigated the ileal effluent of patients with so-called Brooke ileostomies, i.e. patients with an ileum ending in an opening of the abdominal wall, mostly because the colon had to be removed due to colon cancer (Booijink *et al.*, 2010). They showed that the small intestine was characterized by a less diverse and temporarily more fluctuating microbial community than the large intestine (Booijink *et al.*, 2010). Based on community profiles obtained with a phylogenetic microarray, the average community similarity of four patients over 9 days was just 44%. Notably, no *Archaea* were detected in the effluent samples. Although the community of each patient was highly individual, a hypothetical common 'core microbiota' was defined based on these four patients. It comprised bacteria belonging to the genera *Clostridium*, *Enterococcus*, *Oxalobacter*, *Streptococcus* and *Veillonella*.

By comparing small intestinal lumen samples obtained from healthy subjects by means of an extended oral catheter with ileal effluent samples, Zoetendal and co-workers very recently showed that the microbial composition of ileal effluent might rather resemble the community in the jejunum (Zoetendal *et al.*, 2012). They identified bacteria belonging to the *Bacteroidetes*, *Clostridium* cluster XIVa and *Proteobacteria* as typical for the ileum. In line with previous studies (Booijink *et al.*, 2010), they corroborated a lower species diversity and significant temporal fluctuations in community composition, in comparison to the colon or faecal community. Additionally, using metagenomic, metatranscriptomic and metabolite profiling in addition to community profiling, Zoetendal and colleagues (2012) developed an ecological model of the small intestinal microbiota. They found genes coding for carbohydrate phosphotransferase system (PTS) transport mechanisms, central metabolism and biotin biosynthesis being over-represented in the small intestine. Interestingly, these genes were not only abundantly present in the metagenomic libraries, but also showed high-level *in situ* expression, as indicated by metatranscriptomic analysis. Apparently, the small intestine is a habitat where the microbiota has to compete vigorously with the human host for carbohydrates, and consequently microorganisms that possess rapid uptake and conversion mechanisms of simple carbohydrates become enriched.

In a quantitative PCR (qPCR)-based study on the ileal lumen of 17 patients that had to undergo small bowel transplantation, Hartman *et al.* could show that the ileal community before and after surgical closure of an ileostomy differed considerably (Hartman *et al.*, 2009). Before the closure, it was dominated by facultative anaerobes (*Lactobacillus* spp., enterobacteria), while following the closure it was dominated by obligate anaerobes. They concluded that oxygen penetration into the terminal ileum was responsible for the community shift, thereby questioning the relevance, for healthy individuals, of community data obtained with ileostomy patients. Interestingly, the function of the small intestine itself was apparently not affected by this dramatic shift in microbial community composition.

Recent progress on disease-related changes in the small intestinal microbiota has been reviewed expertly by Cotter (2011). For instance, elevated levels of *Bacteroides* spp., *Clostridium leptum*, *Escherichia coli* and *Staphylococcus* spp. and decreased levels of *Bifidobacterium* spp., two other clostridial species and *Faecalibacterium prausnitzii* were detected in duodenal biopsy samples of patients suffering from paediatric coeliac disease (Sokol *et al.*, 2008; Collado *et al.*, 2009;

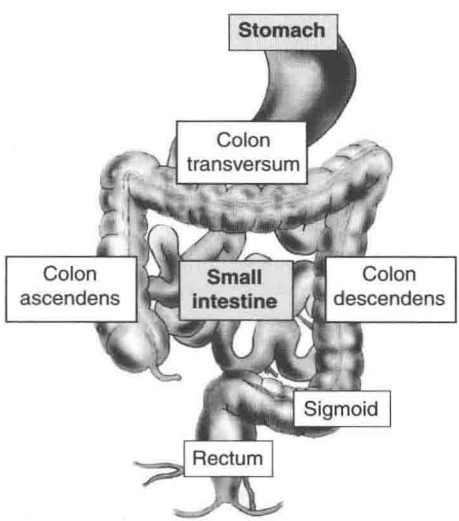
De Palma *et al.*, 2010; Schippa *et al.*, 2010). Lower duodenal levels of *Bifidobacterium catenulatum* were found in patients with irritable bowel syndrome (Kerckhoffs *et al.*, 2009). Finally, lower levels of *F. prausnitzii* and *Ruminococcus gnavus* and elevated levels of *E. coli* and *Roseburia* spp. were found in patients with ileal Crohn's disease (Willing *et al.*, 2009, 2010).

Clearly, more research is needed to differentiate which community changes are causes and which are effects of certain disease states. Moreover, the inventory of the small intestinal species and their longitudinal and transversal spatial distribution is still far from being fully understood. This is, however, a prerequisite to define a 'normal' or 'healthy' microbial community of the small intestine.

1.4 The Microbiota of the Large Intestine

1.4.1 Environmental conditions

The large intestine consists of the caecum, colon (ascending, transverse, descending and sigmoid), rectum and anal canal (Fig. 1.3). In total, it is about 1.5 m long, 6.5 cm in diameter and has a surface area of approximately 1200 cm^2. As in the small intestine, the surface of the colon is covered entirely by mucus under normal conditions. Early studies suggested that the thickness of the colonic mucus layer increased from about 30 μm in the caecum to 90 μm and more in the rectum (Matsuo *et al.*, 1997). However, a very recent analysis has indicated that these values were underestimated and that the mucus layer of the colon might even be up to 450 μm thick (Gustafsson *et al.*, 2012). The morphology of the colonic mucosa differs strongly from that of the small intestine. Permanent folds or villi, as present in the small intestine, are absent. By contrast, the colonic crypts, consisting of absorptive epithelial cells, are lined by a large number of mucus-secreting goblet cells and harbour defensin-producing paneth cells (Metz-Boutigue *et al.*, 2010). The main function of the colonic epithelium is the reabsorption of ions and water. As a result of water absorption, the chyme becomes solid approximately 3–10 h after having entered the large intestine and is then referred to as faeces. No digestive enzymes are secreted by the cells of the large intestine. Breakdown of



Current knowledge

- maximum microbial diversity (collective human GIT microbiota: up to 1800 genera and 15,000 species)
- suggestion of a core metagenome based on gene functions
- definition of three human enterotypes: *Bacteroides*, *Prevotella*, *Ruminococcus*

Key questions

- microheterogeneity of the microbiota along the colon (longitudinal and transversal)
- effect of methodical biases on community composition results
- changes in microbial community composition in intestinal or metabolic diseases: cause or consequence?
- interactions of bacterial, archaeal, viral (phage) and eukaryotic (fungal) microbiomes with each other and with the host

Fig. 1.3. Current knowledge and key questions regarding the microbial ecology of the human large intestine.