

Studying the host-microbiota interaction in the human gastrointestinal tract: basic concepts and *in vitro* approaches

Massimo Marzorati · Pieter Van den Abbeele ·
Sam Possemiers · Jessica Benner · Willy Verstraete ·
Tom Van de Wiele

Received: 4 January 2011 / Accepted: 1 March 2011 / Published online: 20 March 2011
© Springer-Verlag and the University of Milan 2011

Abstract Bacteria in the human gut exceed the number of cells in our body by a 100-fold. At the level of the gastrointestinal epithelium, a constant battle is fought for equilibrium between the microbiota and the human body. These interactions play a key role in many aspects of host health, influencing energy harvest from food, colonization by pathogens, and the immune system, to name but a few. Unfortunately, the study of this host–microbiota interaction *in vivo* is limited by the inaccessibility of the digestive tract. Therefore, *in vitro* technology that focuses on the simulation of this epithelial environment offers an ideal platform with which to conduct mechanistic research that could shed more light on this environment and help explain *in vivo* observations. However, the limitation of currently available tools could yield results with limited reliability for an *in vivo* situation. The aim of this mini-review is to focus on the importance of studying the host–microbiota interaction in the gastrointestinal tract and to evaluate the state of the art of the available *in vitro* techniques. Finally, we aim to identify those missing factors that, if present, would allow the creation of a model that would constitute a better simulation of biofilm formation, i.e. one more closely resembling the *in vivo* situation.

Keywords Gut microbiota · Biofilm · Signal exchange · Shear forces · Microaerophilic conditions

M. Marzorati (✉) · P. Van den Abbeele · S. Possemiers ·
J. Benner · W. Verstraete · T. Van de Wiele
Laboratory of Microbial Ecology and Technology (LabMET),
Ghent University,
Coupure Links 653,
9000 Ghent, Belgium
e-mail: massimo.marzorati@UGent.be

Introduction

The human gastrointestinal tract (GIT) is home to a large number of microorganisms—up to 10^{11} – 10^{12} cells are present per gram of fecal matter in the distal colon—belonging to seven bacterial phyla, of which Firmicutes, Bacteroides and Actinobacteria are the most dominant (Cummings and Macfarlane 1991; Eckburg et al. 2005; Rajilić-Stojanović et al. 2007; Turnbaugh et al. 2007). This extremely complex community is normally functionally stable and yet dynamic in composition. It is considered to play multiple roles related to energy harvesting, preservation of niche functionality, decreasing the colonization and invasion of pathogens, regulation of host fat storage, and immunological induction (Eckburg et al. 2005, and references within; Manning and Gibson 2004; Lebeer et al. 2008). As a consequence, host physiology, in both the healthy and pathological state, is greatly dependent on interaction with this community. Overall, the structure and composition of this ecosystem reflects natural selection at both microbial and host levels to develop a mutual cooperation aimed at functional stability (O’Hara and Shanahan 2006). This interaction occurs mainly at the gut wall level, and it is at this site that the equilibrium is finely tuned. This area of study is often neglected due to, on the one hand, the limited accessibility of the human GIT and, on the other, the intrinsic complexity of recreating *in vitro* conditions relevant to an *in vivo*-like interaction. In this review, we will focus mainly on the importance of studying the host–microbiota interaction in the GIT (with some specific examples) and on the state of the art of the *in vitro* approach, with its relative advantages and drawbacks.

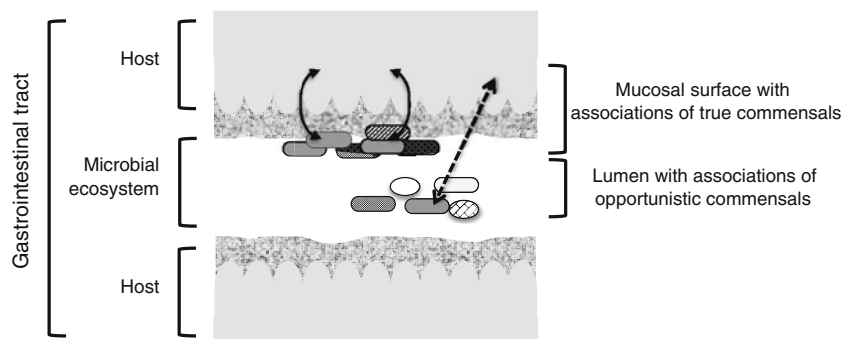
Host–microbiota interaction

The GIT has a form that reflects its specialization in functional anatomy. It is divided into four concentric layers, of which the mucosa—the innermost layer surrounding the lumen—comes in direct contact with the GIT contents. This layer acts as a natural barrier that allows the absorption of nutrients and provides a defense against xenobiotics, digestive enzymes, and bacteria. On top of the epithelial layer, goblet cells secrete mucus, which results in a gel-like film consisting of ca. 95% water, 1–10% glycoproteins and electrolytes, proteins, and antibodies as well as nucleic acids, covering the entire epithelium (Macfarlane et al. 2005). The mucus layer is divided into two layers: an outer layer that is less dense and populated with bacteria, and an inner, denser layer impenetrable to bacteria. Mice lacking a mucus layer, and with consequently an almost sterile zone directly on top of the epithelium, show chronic intestinal inflammation (Hooper 2009). At this level, the commensal microorganisms exert a miscellany of protective, structural and metabolic effects on the intestinal mucosa and are thought to exchange defined signals with the host (microbe-associated molecular patterns, or MAMPs). It has been reported that MAMPs are not invariant generic components of microbial cells, but rather their abundance, structure and signaling properties can be modulated in response to the environmental changes that occur during host colonization (Cheesman and Guillemin 2007). On its side, the host is involved in the accurate interpretation of the micro-environment to distinguish between commensal organisms and possible pathogens, with subsequent precise regulation of the response, mainly through two pattern recognition receptors: toll-like receptors (TLRs) and cytosolic nucleotide oligomerisation domain (NOD) proteins (O’Hara and Shanahan 2007). Such a selection occurs primarily along mucosal surfaces by a host–microbiota cross-talk that leads to modulation of host immunity.

Hence, the equilibrium in this area is based on a deep network of both un-coordinated and coordinated signals, which are conceived to result from an evolutionary stable strategy (i.e., an evolvement in which the potential invader gradually becomes partner; Blaser and Kirschner 2007). This cross signaling can occur both by direct contact (e.g., M cells or dendritic cells) and by secretion of specific molecules (e.g., sIgA, small soluble peptides, DNA; Lebeer et al. 2008). Many of these signals are still unknown. Recently, it has been proposed that this cross-kingdom cell-to-cell signaling involves small molecules, such as hormones, that are produced by the eukaryotes (adrenaline, noradrenaline, ...) and hormone-like chemicals (autoinducer signals 2 and 3 and acyl homoserine lactones) that are produced by bacteria for quorum sensing purposes (Hughes and Sperandio 2008).

In relation to the host–microbiota interaction, it is thus possible to postulate the existence of true commensals, opportunistic commensals and pathogens (Fig. 1). True commensal bacteria are considered to be allowed to have direct contact with the host (adhesion), thereby positively activating innate and adaptive immunity (Corthésy et al. 2007). In exchange, they benefit from a longer retention time in the gut and from a preferential location to influence host physiology. Besides true commensals, the GI tract also hosts a number of bacteria that are opportunistic commensals. They are found mainly in the lumen suspension. The advantage for the host is that they can bring about certain important metabolic processes [e.g., production of short-chain fatty acids (SCFA), vitamins...] in exchange for the possibility of living in an environment with high nutrient availability. When, due to genetic disorders (e.g., inflammatory bowel disease) the immune system becomes poorly regulated, these opportunistic commensals can become a real danger to the host. Finally, negative interactions can also occur as shown by Oliveira et al. (2003): bacteria participate in cancer development and, more specifically, *Listeria monocytogenes* can interfere with the cross-talk between cancer cells and host elements thus modulating

Fig. 1 Conceptual scheme of possible host–microbiota interactions in the gastrointestinal tract (GIT). *Solid arrows* indicate linked signaling between the host and true commensals. The *dashed arrow* represents a different kind of signaling by opportunistic commensals



invasion-associated activities such as cell–cell adhesion or cell–matrix interaction.

Biofilm formation

Relatively little is known about the structure and functions of microbial biofilms in the human GIT. The reason for this is that these structures are located in parts of the digestive tract that are not easily accessible. However, a number of studies have already suggested some peculiar characteristics (Macfarlane and Dillon 2007; Probert and Gibson 2002, and references within). These communities exhibit coordinated multicellular behavior (as do other biofilms in different environments), provide a higher resistance to antibiotics as compared to luminal microorganisms, represent a means of resistance to colonization against potential pathogens, and are directly involved in the stimulation of host immune and hormonal systems. Even if a multitude of species can be retrieved in the biofilms, the capacity to adhere to the mucus layer is not common to all actors present in the GIT. Normally, these structures are dominated by *Bacteroides* spp., *Bifidobacterium* spp., Spirochaetes, Firmicutes (e.g., *Lactobacillus* spp.) and *Fusobacterium* spp. (Probert and Gibson 2002). The latter plays an important role in the early colonization of the mucus layer, acting as a “bridge” and thus favoring the accumulation of the late colonizers (Kolenbrander 2000). From an ecological point of view, living in a biofilm is a selective advantage that allows the microbes to live in a protected niche, to interact directly with the host, and to prolong their stay in the GIT with higher metabolic efficiency (Li et al. 2008; Macfarlane 2008).

Several factors influence the formation of these microbial structures within the GIT. Those that are specific for biofilm formation in the gastrointestinal tract are shown in Fig. 2. Apart from the constant exchange of signals between host and microbiota (as shown in Fig. 1) a special feature of mucosal biofilm is the presence of microaerophilic conditions at the basal side—due to oxygen diffusion from the host blood stream across the epithelium—and of anaerobic conditions on the top. Such

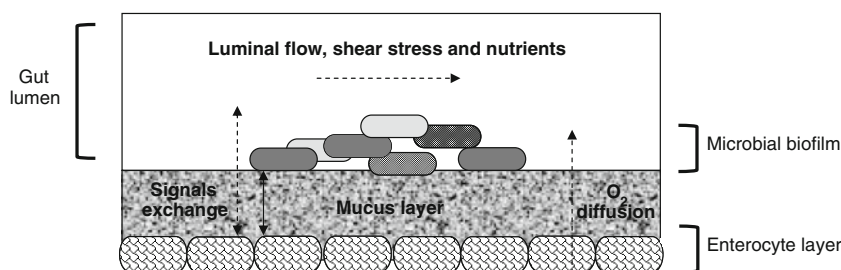
conditions create a favorable niche for first colonizers (i.e., *Fusobacterium* spp), which also act as oxygen scavengers (Probert and Gibson 2002). A second factor of key importance is the presence of shear forces of different intensity (according to the GIT sector), induced by the transit of the bolus and fluids, which exert a mechanical action in shaping the thickness of the biofilm.

According to the developmental model of a gastrointestinal microbial biofilm—as shown by some scientific studies—environmental factors also play a key role in bacterial biofilm formation (Lebeer et al. 2007; Monds and O’Toole 2009). In fact, a number of hierarchically ordered genetic factors control the temporal development of biofilm formation, and these genetic switches are normally activated in response to changes in external stimuli (i.e., shear stress, microbe–microbe interactions, presence of oxygen, host–microbe interactions, etc...).

Gastrointestinal microbial resource management

Several scientific outcomes have confirmed that resident microbiota play a crucial role in maintaining the host health in numerous ways. For instance, probiotic lactobacilli can induce mucin secretion as well as enhance the tight junction functioning (Lebeer et al. 2008, and references within). *Bacteroides thetaiotaomicron* can modulate the expression of genes involved in nutrient absorption, mucosal barrier fortification and xenobiotic metabolism (Hooper et al. 2001). *Bacteroides thetaiotaomicron* can also promote an anti-inflammatory mechanism by the attenuation of pro-inflammatory cytokine expression (PPAR- γ induction linked to the RelA subunit of NF- κ B; Kelly et al. 2004). The same positive effect has been elucidated recently for *Lactobacillus acidophilus* NCFM, which can modulate dendritic cells and T cell functions via the S-layer protein A (Kostantinov et al. 2009). Probiotics also have a role in the production of SCFA, and compounds that can exert a positive effect on gut health (i.e., butyrate; Hamer et al. 2008). Finally, *Bacteroides fragilis* protects animals from experimental colitis induced by *Helicobacter hepaticus*, through the beneficial activity of a single microbial

Fig. 2 Key factors affecting biofilm formation in the human GIT



molecule, polysaccharide A (PSA), which restores the balance between humoral and cell-mediated immunity (Mazmanian et al. 2008).

In the last 20 years, much research effort has focused on the modulation of the colonic microbiota and related metabolic processes by means of, e.g., pre-probiotics, with the aim of improving host health (Gibson et al. 1989; Fooks and Gibson 2002; Manning and Gibson 2004; Sanchez et al. 2009; Possemiers et al. 2010; Marzorati et al. 2010). Such approaches, by analogy with the microbial resource management (MRM) concept (Verstraete 2007; Marzorati et al. 2008), have been defined as gastrointestinal resource management (GRM), i.e., modulation of the gut microbiota and its metabolism with the aim of improving the health of the host (Possemiers et al. 2009, 2010). In this context, less attention has been paid to the host–microbiota interaction at the gut wall level as a key component of our understanding of how different microbial species can contribute to human health. This is probably due to intrinsic limitations in the possibilities of studying these interactions under conditions relevant to an *in vivo* situation. In the next section, we will evaluate the state of the art in this field, listing the advantages and drawbacks of the available techniques.

***In vitro* analysis of the host–microbiota interaction**

To evaluate the potential effect of a specific treatment on the GIT microbial community, and thus to estimate the overall effect on the human host, several possible solutions can be applied: human intervention trials, animal studies, *in vitro* simulation technologies.

In terms of the investigation of host–microbiota cross-talk, *in vivo* studies are the most reliable tool and by far the most relevant. On the other hand, human and animal trials can be extremely complex and expensive, are associated with a number of ethical constraints and, as already mentioned, have limited access to specific areas of the GIT. Besides, animal studies are not always representative of humans. On the contrary, the use of *in vitro* systems to simulate the GIT and to study the mechanistic effect of specific treatments may be a useful and a complementary tool, even if they suffer from the absence of a complete physiological environment (Macfarlane and Macfarlane 2007; Marzorati et al. 2009, 2010). Therefore, *in vitro* technology can be a valuable aid to studying the effect of a given parameter, in excluding specific interfering processes, and explaining *in vivo* observations. Previous studies have validated this approach and shown that, by controlling the nutritional and environmental determinants, it was possible to reproduce *in vitro* microbial communities that can resemble those *in vivo*, with typical microbiota-associated characteristics (Molly et al. 1994; Macfarlane et al. 1998),

although with some differences due to the *in vitro* setup and the lack of the host presence, as shown recently by Van den Abbeele and co-authors (2010). In the last two decades, the need for systems that can better simulate the *in vivo* situation led to the creation of dynamic *in vitro* simulators that attempt to reproduce all or part of the physiological parameters of the luminal environment that could influence the GIT microbial community and its metabolic activity (Molly et al. 1994; Minekus et al. 1999; Macfarlane and Macfarlane 2007). These systems offer good reproducibility in terms of analysis of the luminal microbial community but other aspects, such as biofilm formation and host–microbiota interaction, are limited or neglected.

The study of the host–microbiota cross-talk has to take into account the adhesion of bacteria to the mucus layer covering the gut wall and, at the same time, the mutual effect that bacterial and host cells have on each other following the interaction. Nowadays, these two parameters are usually studied as independent factors. In order to simulate adhesion *in vitro*, Cinquin et al. (2004, 2006) developed a system utilizing cell immobilization in anaerobic continuous-flow cultures. Microbes from fresh fecal samples are immobilized in a mixed gel of gellan and xanthan and the beads are then introduced into a single- or multi-stage chemostat simulating the biofilm that is typically formed in the GIT. Probert and Gibson (2004) proposed a similar device with a framework of mucin beads encased within a dialysis membrane. In addition, Macfarlane et al. (2005) developed a two-stage continuous culture system, simulating the proximal and distal colon, and used sterile porcine mucin gels in small glass tubes to determine how intestinal bacteria colonize and degrade mucus.

Regarding simulation of the host–bacterial interaction, biopsies are a possibility, although they are normally obtained from diseased individuals and thus do not indicate the normal mucosal condition (Macfarlane and Dillon 2007). In order to perform mechanistic studies, the use of cell culture experiments such as Caco-2 (not producing mucus) or HT29 cells (mucus producing if properly triggered) or a combination of the two (Nolleaux et al. 2006) is a common approach. Nevertheless, the output of these reductionist studies is limited by the fact that they are normally conducted using pure cultures or a mix of only a few bacterial strains. In fact, for reasons of cytotoxicity, cell cultures are very sensitive to co-incubation with mixed microbial slurries, thus limiting experimental time to 2 h, maximally 4 h, thus not allowing sufficient time for adaptation of host and microbial metabolism. An evolution of the cell line approach is represented by the three-dimensional organotypic model of human colonic epithelium. This system, which can properly simulate several characteristics normally associated with fully differentiated intestinal epithelia *in vivo* (i.e., tight junction, brush-border

proteins, localized mucin production), has been used, up to now, only to study the invasion of pathogens (Höner zu Bentrup et al. 2006). A final opportunity has been presented by Parlesak et al. (2004), who investigated the interaction between human mononuclear leucocytes and enterocytes when challenged with a single bacterial species using compartmentalized trans-well cell culture systems. In this latter case, the authors concluded that the system was not suited to study the complex properties of the intestinal microbiota over long-term studies.

Limitations of the available approaches

Performing *in vitro* simulations that most closely resemble real conditions is of crucial importance if the results are to be extrapolated to the GIT situation *in vivo*. On the other hand, it must be taken into account that systems characterized by too high complexity may lead to decreased control of environmental parameters, thus making final interpretation of the results too complex. As previously reported, several factors characterize the host–microbiota interface in the GIT. These factors are summarized in Table 1 in relation to the techniques presented in the previous section. It is clear that the available systems have some limitations. First, none of them offers the opportunity to study gut biofilm formation or, at the same time, host–microbiota interactions under continuous simulated conditions. No system that provides the possibility of working with complex microbial communities, such as those present in the GIT, can simulate the presence of a host environment. Moreover, all these systems lack the key point that specifically characterizes the gut mucosal biofilm, i.e., the anaerobic conditions prevailing at the

top of the biofilm and the microaerophilic conditions at the base of it. As a final result, those microorganisms that play a key role as primary colonizers and that act as oxygen scavengers (i.e., *Fusobacterium* spp.) can no longer exert their ecological function. This could have some other implications for biofilm development.

On the other hand, the use of cell lines is limited to the investigation of the effect of pure strains, under static conditions (no shear stress) and for a short time. An enterocyte is exposed, on average, for 48 h during its lifetime when migrating from the crypts to the top of the villi. Besides, no studies with complex microbial communities are yet possible. This is a clear limitation considering that, for instance, it has been shown that Lactobacilli grown in laboratory conditions (as pure strains) and *in vivo* (mixed with other bacteria) exhibit different patterns of gene expression. This is probably related to adaptation to the host environment and microbe–microbe interactions (Lebeer et al. 2008). For instance, it is expected that the GIT environment with its autochthonous microbial community can influence the functioning of lactobacilli adhesins by cell-to-cell communication molecules (Vélez et al. 2007). This possibly leads to limited reliability when studies on the capacity of specific strains are performed using pure cultures.

Conclusions

The study of the *in vivo* functionality of biofilms in the human GIT has, up to now, been limited mainly by the complexity of reaching these microbial structures in the different areas of the digestive tract. In such situations, *in vitro* approaches normally represent an alternative solution for mechanistic studies. However, *in vitro* tests that do not

Table 1 Summary of the key characteristics that an *in vitro* system should have to properly study the host–microbiota interaction, and positioning of the available approaches

Approach	Reference	Key characteristics for a proper <i>in vitro</i> simulation					
		Mucus layer simulation	Shear stress	Bottom microaerophilic conditions	Long-term studies	Working with complex microbial communities	Co-presence of host-microbiota
Gellan and xanthan beads	Cinquin et al. 2006	✓	✓		✓	✓	
Mucin beads in a dialysis membrane	Probert and Gibson 2004	✓	✓		✓	✓	
Mucin gel in glass tubes	Macfarlane et al. 2005	✓			✓	✓	
Caco-2 cells	-						✓ ^a
HT29 cells	-	✓					✓ ^a
3D organotypic models	Höner zu Bentrup et al. 2006	✓	✓				✓ ^a
Trans-well	Parlesak et al. 2004			✓			✓

^a Using pure strains or limited mixes

represent actual *in vivo* complexity may result in non-reliable results (Pedersen and Tannock 1989). To date, a model that can mimic simultaneously several parameters that are of basic importance for biofilm development (shear stress, microbe–microbe interaction, microaerophilic conditions close to the gut wall, host–microbe interaction, etc...) is not yet available. Therefore, *in vitro* models allowing the study of microbial mucus colonization over a longer timeframe with the introduction of the host parameters are urgently needed, and research should point in this direction. The ideal solution would be represented by a new device that could incorporate, at the same time, the presence of complex microbial communities originating from different areas of the GIT (microbiota compartment) and of human cell lines (host compartment), to allow long-term studies and reciprocal host–microbiota adaptation. This could be achieved, for instance, by means of separate compartmentalization of bacteria and cell lines, to avoid direct contact but allowing exchange of metabolites and signals between the two compartments. This appears to be a feasible approach as it is based on the typical structure of a microbial fuel cell (Pham et al. 2009), with the presence of a permeable membrane, as shown by Laube et al. (2000), who developed an *in vitro* system for studying the interaction of xenobiotic metabolism of liver and intestinal microbiota.

Acknowledgments M.M. benefits from an IWT post doctoral grant (OZM 090249). P.V.A. benefits from a FWO-Vlaanderen PhD scholarship, while T.V.W. and S.P. from a postdoctoral grant from FWO-Vlaanderen.

References

- Blaser MJ, Kirschner D (2007) The equilibria that allow bacterial persistence in human hosts. *Nature* 449:843–849
- Cheesman SE, Guillemin K (2007) We know you are in there: conversing with the indigenous gut microbiota. *Res Microbiol* 158:2–9
- Cinquin C, Le Blay G, Fliss I, Lacroix C (2004) Immobilization of infant fecal microbiota and utilization in an *in vitro* colonic fermentation model. *Microb Ecol* 48:128–138
- Cinquin C, Le Blay G, Fliss I, Lacroix C (2006) New three-stage *in vitro* model for infant colonic fermentation with immobilized fecal microbiota. *FEMS Microbiol Ecol* 57:324–336
- Corthésy B, Gaskins HR, Mercenier A (2007) Cross-talk between probiotic bacteria and the host immune system. *J Nutr* 137:781S–790S
- Cummings JH, Macfarlane GT (1991) The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol* 70:443–459
- Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA (2005) Diversity of the human intestinal microbial flora. *Science* 308:1635–1638
- Fooks LJ, Gibson GR (2002) Probiotics as modulators of the gut flora. *Br J Nutr* 88:S39–S49
- Gibson SA, Macfarlane C, Hay S, Macfarlane GT (1989) Significance of microflora in proteolysis in the colon. *Appl Environ Microbiol* 55:679–683
- Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer RJ (2008) Review article: the role of butyrate on colonic function. *Aliment Pharmacol Ther* 27:104–119
- Höner zu Bentrup K, Ramamurthy R, Ott CM, Emami K, Nelman-Gonzalez M, Wilson JW, Richter EG, Goodwin TJ, Alexander JS, Pierson DL, Pellis N, Buchanan KL, Nickerson CA (2006) Three-dimensional organotypic models of human colonic epithelium to study the early stages of enteric salmonellosis. *Microbes Infect* 8:1813–1825
- Hooper LV (2009) Do symbiotic bacteria subvert host immunity? *Nat Rev Microbiol* 7:367–374
- Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI (2001) Molecular analysis of commensal host-microbial relationships in the intestine. *Science* 291:881–884
- Hughes DT, Sperandio V (2008) Inter-kingdom signalling: communication between bacteria and their hosts. *Nat Rev Microbiol* 6:111–120
- Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AG, Pettersson S, Conway S (2004) Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR- γ and RelA. *Nat Immunol* 5:104–112
- Kolenbrander PE (2000) Oral microbial communities: biofilms, interactions, and genetic systems. *Annu Rev Microbiol* 54:413–437
- Konstantinov SR, Smidt H, de Vos WM, Bruijns SC, Singh SK, Valence F, Molle D, Lortal S, Altermann E, Klaenhammer TR, van Kooyk Y (2009) S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. *Proc Natl Acad Sci USA* 105:19474–19479
- Laube B, Winkler S, Ladstetter B, Scheller T, Schwarz LR (2000) Establishment of a novel *in vitro* system for studying the interaction of xenobiotic metabolism of liver and intestinal microflora. *Arch Toxicol* 74:379–387
- Lebeer S, Verhoeven TL, Perea Vélez M, Vanderleyden J, De Keersmaecker SC (2007) Impact of environmental and genetic factors on biofilm formation by the probiotic strain *Lactobacillus rhamnosus* GG. *Appl Environ Microbiol* 73:6768–6775
- Lebeer S, Vanderleyden J, De Keersmaecker SC (2008) Genes and molecules of lactobacilli supporting probiotic action. *Microbiol Mol Biol Rev* 72:728–764
- Li XJ, Yue LY, Guan XF, Qiao SY (2008) The adhesion of putative probiotic lactobacilli to cultured epithelial cells and porcine intestinal mucus. *J Appl Microbiol* 104:1082–1091
- Macfarlane S (2008) Microbial biofilm communities in the gastrointestinal tract. *J Clin Gastroenterol* 42(Suppl 3):S142–S143
- Macfarlane S, Dillon JF (2007) Microbial biofilms in the human gastrointestinal tract. *J Appl Microbiol* 102:1187–1196
- Macfarlane GT, Macfarlane S (2007) Models for intestinal fermentation: association between food components, delivery systems, bioavailability and functional interactions in the gut. *Curr Opin Biotechnol* 18:156–162
- MacFarlane GT, MacFarlane S, Gibson GR (1998) Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colon. *Microb Ecol* 35:180–187
- Macfarlane S, Woodmansey EJ, Macfarlane GT (2005) Colonization of mucin by human intestinal bacteria and establishment of biofilm communities in a two-stage continuous culture system. *Appl Environ Microbiol* 71:7483–7492
- Manning TS, Gibson GR (2004) Microbial-gut interactions in health and disease: prebiotics. *Best Pract Res Clin Gastroenterol* 18:287–298
- Marzorati M, Wittebolle L, Boon N, Daffonchio D, Verstraete W (2008) How to get more out of molecular fingerprints: practical tools for microbial ecology. *Environ Microbiol* 10:1571–1581

- Marzorati M, Possemiers S, Verstraete W (2009) The use of the SHIME-related technology platform to assess the efficacy of pre- and probiotics. *Agro Food Ind Hi-Tech* 20:S50–S55
- Marzorati M, Verhelst A, Luta G et al. (2010) Modulation of the human gastrointestinal microbial community by plant-derived polysaccharide-rich dietary supplements. *139*:168–176
- Mazmanian SK, Round JL, Kasper DL (2008) A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 453:620–625
- Minekus M, Smeets-Peeters MJE, Bernalier A, Marol-Bonnin S, Havenaar R, Marteau P, Alric M, Fonty G, Huis in 't Veld JHJ (1999) A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. *Appl Microbiol Biotechnol* 53:108–114
- Molly K, Vande Woestyne M et al (1994) Validation of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) reactor using microorganism-associated activities. *Microb Ecol Health Dis* 7:191–200
- Monds RD, O'Toole GA (2009) The developmental model of microbial biofilms: ten years of a paradigm up for review. *Trends Microbiol* 17:73–87
- Nollevaux G, Devillé C, El Moualij B, Zorzi W, Deloyer P, Schneider YJ, Peulen O, Dandriofosse G (2006) Development of a serum-free co-culture of human intestinal epithelium cell-lines (Caco-2/HT29-5M21). *BMC Cell Biol* 7:20
- O'Hara AM, Shanahan F (2006) The gut flora as a forgotten organ. *EMBO Rep* 7:688–693
- O'Hara AM, Shanahan F (2007) Gut microbiota: mining for therapeutic potential. *Clin Gastroenterol Hepatol* 5:274–284
- Oliveira MJ, Van Damme J, Lauwaet T et al (2003) Beta-casein-derived peptides, produced by bacteria, stimulate cancer cell invasion and motility. *EMBO J* 22:6161–6173
- Parlesak A, Haller D, Brinz S, Baeuerlein A, Bode C (2004) Modulation of cytokine release by differentiated CACO-2 cells in a compartmentalized coculture model with mononuclear leucocytes and nonpathogenic bacteria. *Scand J Immunol* 60:477–485
- Pedersen K, Tannock GW (1989) Colonization of the porcine gastrointestinal tract by lactobacilli. *Appl Environ Microbiol* 55:279–283
- Pham H, Boon N, Marzorati M, Verstraete W (2009) Enhanced removal of 1,2-dichloroethane by anodophilic microbial consortia. *Water Res* 43:2936–2946
- Possemiers S, Grootaert C, Vermeiren J, Gross G, Marzorati M, Verstraete W, Van de Wiele T (2009) The intestinal environment in health and disease—recent insights on the potential of intestinal bacteria to influence human health. *Curr Pharm Des* 15:2051–2065
- Possemiers S, Marzorati M, Verstraete W, Van de Wiele T (2010) Bacteria and chocolate: a successful combination for probiotic delivery. *J Food Microbiol* 141:97–103
- Probert HM, Gibson GR (2002) Bacterial biofilms in the human gastrointestinal tract. *Curr Issues Intest Microbiol* 3:23–27
- Probert HM, Gibson GR (2004) Development of a fermentation system to model sessile bacterial populations in the human colon. *Biofilms* 1:13–19
- Rajilić-Stojanović M, Smidt H, de Vos WM (2007) Diversity of the human gastrointestinal tract microbiota revisited. *Environ Microbiol* 9:2125–2136
- Sanchez JI, Marzorati M, Grootaert C, Baran M et al (2009) Arabinoxylan-oligosaccharides (AXOS) affect the protein/carbohydrate fermentation balance and microbial population dynamics of the Simulator of Human Intestinal Microbial Ecosystem. *Microb Biotechnol* 2:101–113
- Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI (2007) The human microbiome project. *Nature* 449:804–810
- Van den Abbeele P, Grootaert C, Marzorati M, Possemiers S et al (2010) Microbial community development in a dynamic gut model is reproducible, colon region specific, and selective for Bacteroidetes and *Clostridium* cluster IX. *Appl Environ Microbiol* 76:5237–5246
- Vélez PM, De Keersmaecker SC, Vanderleyden J (2007) Adherence factors of *Lactobacillus* in the human gastrointestinal tract. *FEMS Microbiol Lett* 276:140–148
- Verstraete W (2007) Microbial ecology and environmental biotechnology. *ISME J* 1:4–8