Mol. Nutr. Food Res. 2011, *55*, 969–978 DOI 10.1002/mnfr.201000589

REVIEW

Bacterial biofilms associated with food particles in the human large bowel

Amy S. Van Wey[1,2], *Adrian L. Cookson*[1,3], *Nicole C. Roy*[1,3], *Warren C. McNabb*[1,3], *Tanya K. Soboleva*[4] *and Paul R. Shorten*[1,2]

[1] Riddet Institute, Massey University, Palmerston North, New Zealand
[2] AgResearch, Ruakura Research Centre, Hamilton, New Zealand
[3] AgResearch, Grasslands Research Centre, Palmerston North, New Zealand
[4] Ministry of Agriculture and Forestry, Wellington, New Zealand

Bacteria within the gastro-intestinal tract affect host function via production of short-chain fatty acids and synthesis of vitamins. Additionally, the commensal enteric bacteria modulate the immune system and provide protection from potentially pathogenic bacteria. Only recently heterogeneous bacterial biofilms were found to be associated with food particles within the intestinal tract. There are a number of studies investigating the formation and function of pathogenic and single-species biofilms, though few studies have investigated the dynamics of multispecies biofilms, especially with regard to food/microbial/host interactions. The scope of this review is to discuss the current knowledge of bacterial biofilms associated with food particles in the human large bowel, examine the established mathematical models depicting bacterial attachment, and elucidate key areas for further research.

Received: November 23, 2010
Revised: March 9, 2011
Accepted: March 17, 2011

Keywords:
Attachment / Diet / DLVO / Microbial / Models

1 Introduction

In the developed countries, some individuals are experiencing personal health deterioration due to busy and often sedentary lifestyles that include an overconsumption of energy-dense, nutritionally poor convenience foods [1]. This is evident by the prevalence of obesity, cardiovascular diseases, and diabetes in developed countries [2–4]. The decline of health, combined with new developments in nutritional science and media attention to the link between diet and health, has resulted in a higher demand for foods that improve human health [1]. The global market for functional foods, foods that confer health benefits beyond basic nutrition, has increased from US$33 billion in 2000 to US$74 billion in 2005, with global sales expected to reach US$167 billion in 2010 [1].

Many functional foods target the growth of intestinal bacteria since it has become widely accepted that the maintenance of commensal bacterial populations is necessary for intestinal (and overall) health. Owing to the beneficial effects of commensal bacteria, the formulation and utilization of probiotics, prebiotics, and synbiotics have been at the forefront of nutritional research [5]. Bacteria within the large bowel have been found to affect host function by producing short-chain fatty acids (SCFA) [6–8], synthesizing vitamins [7, 9–12], modulating the immune system [13–16], and acting as colonization inhibitors by potential pathogenic bacteria [17–19].

The surface area of the human bowel (400 m^2) combined with an ideal temperature and an abundance of nutrients provide an environment in which anaerobic and facultative anaerobic bacteria thrive [20]. It is estimated that there are 10^{14} bacteria cells within the digestive tract, which is 10 times the number of cells that constitute the human anat-

Correspondence: Dr. Adrian L. Cookson, Grasslands Research Centre, AgResearch Ltd., Tennent Drive, Private Bag 11008, Palmerston North 4442, New Zealand
E-mail: adrian.cookson@agresearch.co.nz
Fax: +64-6-351-8032

Abbreviations: AB, acid–base; **BSH,** bile salt hydrolase; **DLVO,** Derjaguin–Landau–Verwey–Overbeek; **EPS,** extracellular polymeric substances; **GI,** gastro-intestinal; **LW,** Lifshitz–van der Waals; **XDLVO,** extended DLVO omy [21]. The short digestive time (2–4 h) causes a rapid flow of material through the small bowel, inhibiting bacterial colonization [22], whereas the digestive time within the large bowel, 1–3 days, provides an ideal environment for bacterial growth and fermentation (see Fig. 1). There are over 1000 distinct bacterial species [23] with 30–40 species comprising up to 99% of the total population [24]. This may be an underestimate since only 10–50% of the bacteria are cultivable [25–27]. Current metagenomic studies have shown that while the bacterial population of the adult human intestinal tract is heterogeneous and individual specific (due to health, diet, age, and environment), their metabolism and overall function are similar [27] and are unique to the intestinal environment [28].

Given the prevalence of bacterial biofilms associated with food particles in ruminants, it was assumed that bacteria formed biofilms on digesta within the human intestinal tract, and in particular within the cecum and colon [29]. However, the first reports of bacterial biofilms associated with food particles in human fecal matter were described by Macfarlane et al. in 1997 [30–32]. Owing to the difficulty in studying digesta within the human cecum and colon, little is known about the mechanisms used for bacterial attachment or growth on food particles in vivo. The majority of our knowledge has been gained from in vitro experimentation utilizing fecal inocula or single bacterial species. However, studies of the intestinal contents of sudden death victims have shown higher levels of SCFA in the proximal colon (cecum and ascending colon) indicating carbohydrate fermentation [33, 34]. Although biofilms constitute only 5% of the bacterial population in human fecal samples [30], high levels of acetate within the proximal colon, which is readily produced by heterogeneous bacterial biofilms [22, 30, 35], imply that either bacterial biofilms are prolific in this site or that the formation of a biofilm leads to significant production of acetate.

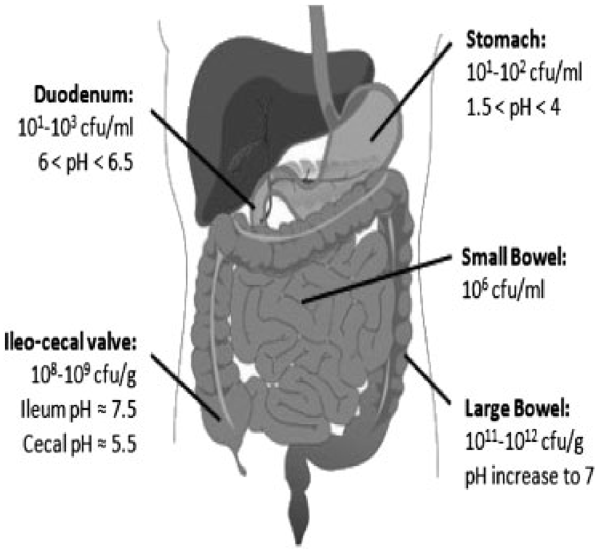

Figure 1. Bacterial counts in the GI tract, including the stomach [22], duodenum [116], and small bowel [15] (colony forming units/mL luminal contents) and at the ileo-cecal valve [22] and within the large bowel (colony forming units/g luminal contents) [117]. Image modified from Wikimedia Commons.

With the possibility of biofilms being prevalent in the large bowel, extensive research into the effects and functionality of digesta-associated biofilms is necessary. To assist in this endeavor, the scope of this review is to discuss the current knowledge of bacterial biofilms associated with food particles in the human large bowel, examine the established mathematical models depicting bacterial attachment, and elucidate key areas for further research.

2 Diet

Until recently, human newborn intestinal tracts were believed to be sterile at birth and bacterial colonization was influenced by the mode of delivery (cesarean versus vaginal delivery), premature delivery and exposure to intensive care units, feeding (breast fed versus formula fed), and sanitation [25, 36–38]. While these factors influence the bacterial population in neonates, recent research suggests that bacteria inhabit the fetal intestinal tract in utero [39–41] and that breast milk supplies both oligosaccharides and commensal bacteria that have prebiotic and probiotic effects, respectively, in the infant [37, 38].

While the metabolic requirements for most of the enteric bacteria and the metabolites produced by these bacteria are unknown [42], the current metagenomic studies of bacteria from fecal samples have shown that the intestinal bacteria of adult, weaned and unweaned children primarily function as carbohydrate transporters and metabolizers [27]. There was a notable difference between the weaned children/adult microbiome, the collective genomes of the microbiota, with that of the unweaned infant microbiome, which may be attributed to the bacteria's role in transport and uptake of simple sugars from breast milk.

A recent study comparing the intestinal bacteria of European children, fed a Westernized diet high in sugar, animal fat and energy dense foods, and the intestinal bacteria of rural African children, fed a diet high in plant polysaccharides, demonstrated significant differences in the bacterial population once breastfeeding was replaced by solid foods [43]. The ratio of *Firmicutes* to *Bacteroidetes* were particularly different; *Bacteroidetes* such as *Prevotella* and *Xylanibacter* with cellulolytic and xylanolytic plant polysaccharide degradation genes being most prevalent in the rural African children. Similar disparities in the ratio of *Firmicutes* to *Bacteroidetes* have been recorded in obese versus lean individuals [44]. De Filippo et al. proposed that the ratio of *Firmicutes* to *Bacteroidetes* may be used as an indicator for future obesity risk and that this disparity may result from diets rich in fat, animal protein, and sugar rather than diets high in plant carbohydrates and resistant starch [43].

The dominant phyla in fecal samples are *Firmicutes* and *Bacteroidetes* [27]. *Firmicutes*, in particular *Ruminococcus* species from *Clostridium* cluster IV, have been found to adhere to and form biofilms on particulates such as plant polysaccharides, resistant starch, and mucin [35, 45]. This difference in substrate association and utilization by *Firmicutes* and *Bacteroidetes* was also reported by Mirande et al. who compared dietary fiber degradation by *Bacteroides xylanisolvens* SB1A and *Roseburia intestinalis* XB6B4 [46]. It is surmised that *Firmicutes* are more efficient at fermenting insoluble carbohydrates [44], whereas many *Bacteroides* species, which demonstrate substrate versatility [24, 35], may rely on soluble oligosaccharides and polysaccharides released by the breakdown of insoluble polysaccharides [45] in a competitive environment. The ability of some bacterial species to metabolize the products produced by or released by another species is called microbial cross-feeding, which attributes to the heterogeneity of bacterial biofilms.

Currently, most studies investigating the influence of diet on the commensal enteric bacteria have primarily investigated the effects of prebiotics or synbiotics [47]. Additionally, these studies focus on how particular food components affect specific bacterial species rather than changes in phylum distribution. It is important to note that fecal samples are often used to enumerate and distinguish bacterial species in human studies. This, however, may not be an accurate representation of the bacterial population in specific areas of the bowel.

The information obtained from sudden death victims has been helpful in confirming the degradation of indigestible carbohydrates within the colon, as well as in determining the metabolites produced by the commensal bacteria [33, 34]. Additionally, these studies have shown that the microbiota is capable of proteolysis, which is evidenced by the occurrence of branch-chained fatty acids in the human distal colon [48]. Therefore, it is reasonable to expect that the bacterial populations associated with the digesta will change over time and may be determined by the substrates available within the digesta. The bacterial population in fecal samples may more aptly reflect the bacterial population within the transverse and descending colon rather than the cecum and ascending colon, where carbohydrate fermentation primarily takes place. This has been substantiated by a study comparing the cecal and fecal bacteria of healthy humans. *Bacteroides* and *Clostridium* groups represented 44% of the fecal bacterial rRNA and only 13% of the cecal bacterial rRNA. Additionally, *Escherichia coli* and the *Lactobacillus–Enterococcus* group represented 50% of the cecal and 7% of the fecal bacteria rRNA [49].

3 Bacterial biofilm formation

Biofilms are distinguished from adherent microcolonies by the evidence of a slime coating the bacterial consortia. This slime or extracellular polymeric substances (EPS) can be comprised of polysaccharides, proteins, DNA, lipids and humic excretions that are associated primarily through ionic interactions [50]. Additionally, 90–99% of the biofilm water content lies within the EPS [51]. The water within the biofilm is not uniformly distributed due to the spatial distribution of bacteria and the overall structure of the biofilm.

In any environment, biofilm formation follows a five-step process: (i) surface conditioning, (ii) initial, reversible attachment to the conditioning film, (iii) irreversible attachment, (iv) accumulation or growth, and (v) detachment [52].

3.1 Surface conditioning

Surface conditioning is the adsorption and accumulation of small molecules to a surface. Surface conditioning has been observed primarily in studies of bacteria in marine environments and bacteria associated with biomaterials. Prior to bacterial attachment, water, ions, proteins, lipids, polysaccharides, and humic secretions become attached to and accumulate on biomaterials and tissues within humans [50]. Conditioning alters the surface and impacts the affinity of bacteria to the surface [53, 54]. Currently, information pertaining to specific diets and their influence on surface conditioning is lacking in the literature. However, it is probable that surface conditioning occurs on food particles within the gastro-intestinal (GI) tract due to the complexity of the digesta, which includes partially degraded or indigestible food particles, digestive enzymes, epithelial cells, mucus, and microbial metabolites. Additionally, in vitro studies have provided evidence that ions, such as calcium and magnesium [55], and glycoproteins, such as fibronectin [56], may improve bacterial attachment.

3.2 Initial and irreversible attachment

Initial and irreversible attachments are not necessarily distinguishable phases in biofilm development. Initial attachment is thought to be affected by the hydrophobicity, cell size, and the electric charge of both the bacterial cell and conditioned surface [57] as well as environmental factors, such as receptor sites (such as glycoproteins) [50], nutrient availability, fluid dynamics, and competing bacteria. These mechanisms may be relatively weak and detachment may occur. Thus, the subsequent or simultaneous adherence of proteins found on cellular membranes and ends of filamentous cell appendages, such as fimbriae or pili, as well as the secretion of EPS, binds the bacteria irreversibly to the substratum [50].

Given the difficulty of human studies of intestinal digesta movement, insights have been gained from animal experiments. Lentle and Janssen suggested that laminar mixing (in the small bowel) may result from simultaneous circular and longitudinal contractions [58]. These contractions may be able to generate radial mixing with the flow dependent upon the viscosity of the digesta, in that increased amounts of indigestible carbohydrate reduces radial mixing. Little is known about the mixing of contents within the human cecum, though it is reasonable to assume that the mixing of the digesta is minimal given that indigestible carbohydrates make up the majority of the digestive material. Moreover, as the digesta progresses through the digestive tract it changes from a pseudoplastic fluid (in the small bowel) to a solid (in the transverse colon) due to absorption of water [59]. Dehydration of the digesta and reduced frequency of radial constriction and peristalsis result in little to no churning of the digesta in the transverse and distal colon [60]. This implies that the motility of non-flagellated/non-pilated bacteria in the large bowel is largely due to diffusion rather than fluid transport.

Generally, both the bacteria and the conditioned substratum are negatively charged [50], although surface conditioning by ions may create a neutrally charged substratum [55]. A combination of van der Waal and hydrophobic forces, and possibly chemical interactions with closely associated cells (electrostatic, hydrogen, and covalent bonding) may allow the bacterial cells to overcome repulsive force from surfaces [57, 61, 62]. In addition to environmental factors and molecular mechanisms, flagella enable bacteria to overcome repulsion, although continued motility after attachment can hinder biofilm formation [61, 62].

Given the diversity of environmental factors, many bacteria have the ability to express different adhesins. Biofilm formation by Gram-negative bacteria such as *Enterobacteriaceae* is facilitated by proteins on either curli fimbriae or Type 1 fimbriae, depending on environmental factors [61, 63, 64]. In experiments using pediatric intestinal tissue, the adherence of enteropathogenic *E. coli* required the direct contact of an outer surface protein, intimin, with the mucosa [65]. Enteropathogenic *E. coli* secretes several effector proteins into the host cell and embeds a secreted protein, translocated intimin receptor (Tir), into the host cell membrane, generating a receptor for intimin.

While the use of flagellum and pili may be essential for the attachment of bacteria to human tissue, Kurokawa et al. reported that motility is not required for commensal enteric bacteria to persist within the lumen of the colon [27]. They suggested that flagellated bacteria, which are immunogenic, may be easily targeted and eliminated by the immune system.

3.2.1 Bacterial attachment to food particles

The attachment mechanisms used by pathogenic bacteria have been studied extensively and there are numerous studies focusing on colonization inhibition via surface conditioning of biomedical devices. However, very little is known about the mechanisms used by commensal bacteria to attach to resistant starch, indigestible non-starch polysaccharides, oligosaccharides, proteins, and lipids within the intestinal lumen or how surface conditioning effects attachment. The particular mechanism required for attachment to resistant starch has been elucidated for only a few strains of enteric bacteria. Surface cellular proteins appear to facilitate attachment to particular substrates, e.g. resistant starch, which has been substantiated by studies involving *Bacteroides thetaiotaomicron* [66, 67], four additional intestinal *Bacteroidetes* [68], *Bifidobacteria adolescentis*, and *Bifidobacteria pseudolongum* [69].

The mechanisms used to bind the enteric microbiota to either milk oligosaccharides, lipids, or proteins are not well understood. Given the high proportion of carbohydrates in the digesta [70] and the emphasis on carbohydrate metabolism by the enteric bacteria [27], it is not surprising that studies have focused on the utilization of resistant starch and dietary fiber by the intestinal microbiota. However, oligosaccharides, present at 10–20 g/L in human milk, are a primary substrate for *Bifidobacterium* within the infant intestinal tract [71]. In the infant large bowel, bacteria may attach to and metabolize available milk oligosaccharides. Therefore, the inclusion of milk oligosaccharides in further studies investigating the attachment mechanisms of enteric bacteria to carbohydrates would be invaluable and may provide additional insights into the formation of bacterial biofilms in the lumen of the infant GI tract.

Although the availability of protein in the large bowel is approximately one-fifth that of carbohydrates [70], enteric bacteria metabolize protein within the distal bowel. Additionally, it has been hypothesized that the metabolites from proteolytic fermentation may increase the risks of colon cancer and inflammatory bowel disease [42, 72]. Thus, there is a need to both ascertain which species metabolize protein and determine the binding mechanisms used by the enteric bacteria.

Although lipid metabolism primarily takes place in the small bowel approximately 5–10% (0.3–0.6 g/day) of bile acid escapes reabsorption in the distal ileum [73, 74]. There is evidence to support that, in humans, deconjugation of bile acids begins in the small bowel [75] and continues in the cecum and colon [76–79]. Studies detected bile salt hydrolase (BSH) activity, the ability to deconjugate bile salts, in *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Clostridium*, and *Bacteroides* spp. [74]. The function of BSH activity is still unknown, but it has been suggested that the bacteria incorporate cholesterol and/or bile into the bacterial cell membrane which could strengthen the membrane and alter the charge of the bacterium. Such modifications of the bacterial cell membrane may increase their ability to survive areas of the GI tract that have a low pH. Although the fecal bacteria have the ability to hydrolyze bile salts, our current understanding of the human large bowel microbiome shows that the genes required for lipid transport and metabolism are under-represented [27]; however, this is likely due to the relatively low levels of bile acids that enter the colon and that only ~0.0001% of the total colonic microbiota are estimated to be capable of converting primary bile acids to secondary bile acids [78]. Regardless, if indeed BSH activity results in a change of the net charge of the bacterial cell membrane, this may have an impact on the attachment process of these bacteria to food particles within the large bowel. Further studies are needed.

3.2.2 Theoretical models for bacterial attachment

In vitro and in vivo experimentation is complemented by mathematical modeling. There are three accepted theories that describe attachment of charged molecules or cells to a substratum in a liquid environment: Derjaguin–Landau–Verwey–Overbeek (DLVO), Lifshitz–van der Waals (LW) acid–base (AB) approach to thermodynamic theory and extended DLVO (XDLVO) theory [80].

DLVO theory was originally developed to describe the behavior of colloidal particles with a surface in a liquid environment. DLVO theory assumes that the total interaction energy, or energy of adhesion, is dependent upon the separation distance between the surface and particle and is described as the sum of energies from LW interactions and electrostatic repulsion [81]. DLVO theory was adopted to explain the attachment of bacteria, which are similar in size to colloids, to a surface as this process can follow two steps: reversible and irreversible attachment [81, 82]. However, not all bacteria appear to follow this two-step process. Studies have shown that the production of EPS and flagellum may also influence bacterial attachment at relatively long ranges (100 nm) [55, 83–86], which is not accounted for in the DLVO theory. According to DLVO theory, bacterial attachment should not occur at such distances but bacteria with EPS and/or flagellum are readily able to attach at distances up to 100 nm [55, 83–86]. Furthermore, DLVO does not account for attraction due to hydrophobic interactions, which can be 10–100 times stronger than those from LW [87, 88]. The LW-AB thermodynamic theory for bacterial attachment was developed to overcome these shortcomings.

Van Oss et al. [89] proposed the LW-AB thermodynamic theory in which the AB component accounted for the observation that hydrophobic bacterial cells readily attach to a hydrophobic substratum and that hydrophilic bacterial cells prefer a hydrophilic substratum. The thermodynamic LW-AB approach considers that the total free energy of adhesion is the sum of LW (apolar) interactions and AB (polar) interactions (i.e. all possible electron-donor/electron-acceptor interactions) [90] at the substratum–liquid interface, substratum–bacterial interface, and bacterial–liquid interface [80]. The AB interactions are often referred to as hydrophobic/hydrophilic interactions due to potential hydrogen bonding. The LW-AB theory does not consider the distance dependence of the LW or AB interactions nor does it account for reversible attachment, as the model requires a cell–substratum interface.

Given the respective merits of DLVO and LW-AB theory, Van Oss extended the DLVO theory by incorporating AB interactions in the total energy of bacterial–substratum interactions [91]. Therefore, XDLVO theory can be considered a combination of DLVO and LW-AB theories [80].

There is increasing evidence to suggest that all three theories do not adequately describe bacterial attachment within the context of the human GI tract [55, 56, 80, 87, 92–94]. Studies have shown that LW-AB and XDLVO theories are more accurate than DLVO, which may be due to the incorporation of AB interactions in both the XDLVO and LW-AB models. As mentioned earlier, hydrophobic interactions can be 10–100 times stronger than those of LW interactions when the cell–substratum distance is within 5 nm [87, 88]. Additionally, experiments studying bacterial attachment found that surface conditioning by particular ions, such as magnesium and calcium, can alter the charge of the substratum so that electrostatic and LW interactions have an insignificant influence on bacterial attachment [55, 87, 93, 94]. Thus, the incorporation of AB interactions in the theoretical models of bacterial attachment appears to be necessary [95].

Bacterial attachment is a very complex process. While these models describe aspects of the physicochemical properties of bacterial attachment, they do not, however, incorporate biological factors. The lack of inclusion of biological factors in the above models may contribute to the discrepancy between model predictions and experimental observations. Currently, these models are used to describe the attachment of bacteria to surfaces in a variety of environments. A critical analysis of the agreement of XDLVO and LW-AB theories with experiments may give valuable insights. Given the complexity of the various environments in which bacteria grow and the environmental specific attachment methods employed by bacteria, a universal model may not be feasible or appropriate.

3.3 Biofilm growth and detachment

Once irreversible attachment to a substratum has been established, biofilm maturity is facilitated by the generation of daughter cells [96] and the recruitment of bacteria from the surrounding medium. Both cellular proteins and the production of EPS appear to help bind the bacteria to the substratum and aid in cell-to-cell adherence [96, 97]. Biofilm-associated protein homolog, Bap, and accumulation-associated protein, Aap, are surface proteins that are thought to mediate biofilm formation via cell-to-cell contact with some *Staphylococcus* strains [98]. In oral *Streptococcus*, bacterial cell–cell aggregation or coaggregation is thought to be attributed to either lectin–saccharide or protein–protein interactions [99].

Presently, the role of the EPS remains equivocal. For example, the components of the EPS can aid binding of bacteria to conditioned surfaces [100–102], be necessary for biofilm formation by providing structural stability [100, 102, 103], promote both aggregation and coaggregation by either providing receptor sites to which bacteria can bind [100, 102–104] or by supplying nutrients for utilization by non-competing bacteria [105], and act as a storage receptacle for nutrients to abate variation in nutrient availability [105]. Given the diverse observations, it is likely that the EPS is multifunctional and that functional components will vary depending on the types of bacteria associated within the biofilm and environmental factors.

Biofilms do not experience unrestricted growth. The development of the biofilm is restricted by nutrient availability, removal of waste, interactions with the immune system, and quorum sensing.

3.3.1 Nutrients and growth

It is accepted that the mechanism employed for nutrient transport into the biofilm is diffusion [106]; thus, nutrient gradients develop within the biofilm. This, in turn, affects the growth rate of the bacteria within the biofilm. It is well documented that bacteria within biofilms, primarily near a non-organic substratum, exhibit a quiescent state brought on by the general stress response from nutrient and oxygen deprivation, changes in pH, and the buildup of toxic by-products [53], whereas bacteria on the outer layer of the biofilm, where nutrients are easily accessible, express a growth and metabolic rate similar to planktonic (free floating) bacteria [107]. However, this is not necessarily the case for biofilms attached to food particles because the substratum acts as a substrate. In this scenario, bacteria closely associated with the substratum and those on the periphery (closest to the intestinal lumen) have more access to nutrients (Sandra MacFarlane, personal communication) and thus would demonstrate the greatest growth rate.

3.3.2 Quorum signaling: structure, virulence, and detachment

Bacteria within biofilms demonstrate a number of phenotypic differences from their planktonic counterparts. One such difference is the production of signaling molecules by both Gram-negative and Gram-positive bacteria, which appear to be regulated by bacterial cell population density [108]. Acyl-homoserine lactones and post-translationally processed peptides, in Gram-negative and Gram-positive bacteria, respectively, have been implicated in the regulation of biofilm development, biofilm structure, virulence, and cell detachment (the fifth stage of biofilm development) [109].

Current studies exploring the mechanisms of quorum sensing in bacterial biofilms have focused on pathogenic bacteria. Biofilm-associated *Vibrio cholerae* have been shown to be 1000-fold more resistant to acid than planktonic bacteria [110], allowing them to survive the low pH of the stomach. *V. cholerae* biofilm formation is initiated by the quorum-sensing regulator LuxO while maintenance is dependent upon the HapR regulator. Once within the bowel, detachment from the biofilm, which is regulated by HapR, is necessary for dispersal and maximal colonization of the small bowel [110].

Quorum sensing appears to be necessary for differentiation, maintenance, and detachment of *Pseudomonas aeruginosa* biofilms. Though this bacterium is not found within the GI tract, it has been well studied and many of the architectural and biofilm-related processes of *P. aeruginosa* may be similar to intestinal bacteria. There are two recognized cell-to-cell signaling systems in *P. aeruginosa*: *lasI–lasR* and *rhlR–rhlI* [111]. The *lasI–lasR* system initiates biofilm formation via the synthesis of the quorum-sensing signal *N*-3-oxododecanoyl-L-homoserine lactone ($N3OC_{12}$-HSL), whereas the *rhlR–rhlI* system influences biofilm structure, maintenance, and detachment [112–114].

E. coli K-12 may rely on the EPS component colanic acid for structural differentiation. Strains deficient in colanic acid produced densely packed, thin (approximately two-cell depth), uniform biofilms similar to *P. aeruginosa* biofilms lacking in $N3OC_{12}$-HSL. Biofilms with colanic acid formed complex, three-dimensional pillars (26 μm high) [115]. Given the similarity of colanic-acid-deficient *E. coli* biofilms and the *P. aeruginosa lasI* mutant biofilms, Danese et al. hypothesize that quorum sensing may play a role in colanic acid production [115].

Presently, the specific processes involved in the structural formation and detachment of commensal enteric bacterial biofilms attached to food particles have yet to be ascertained. However, it is likely that both quorum sensing and nutrient availability will have a significant influence on both the structural formation and detachment of bacterial biofilms attached to digesta.

4 Concluding remarks

As our knowledge of food/bacteria/host interactions increases, it is becoming clear that there is enormous potential in the development of functional foods. With the increasing incidence of intestinal-related conditions, allergies, and food intolerances, there is a growing awareness by the public for alternative methods to improve general health. To maximize the potential health benefits from functional foods, it is essential that we develop a deeper understanding of (i) how diet impacts bacterial attachment to the digesta; (ii) how diet impacts the bacterial colonization of the large bowel; and (iii) how bacteria work in a consortium to degrade food particles.

Until recently, research has focused on pathogenic bacteria, most of which are associated with the intestinal epithelium rather than the digesta. Additionally, studies of the commensal bacteria associated with the intestinal lumen and digesta have concentrated on enumeration, classification, determination of preferred substrates of known species and potential metabolites released via carbohydrate fermentation, and to a lesser extent protein metabolism and bile acid hydrolysis.

It is clear that this area of research is still in its infancy. All of the species associated with the GI tract, and in particular associated with the digesta, have yet to be identified. While the enteric microbiota is capable of metabolizing a variety of dietary and endogenous compounds, the mechanisms required for the attachment to these substrates have only been determined for a few bacterial strains and primarily to resistant starch. When testing the current theoretical models for bacterial attachment, there is clear evidence that these models do not accurately reflect bacterial attachment in this dynamic system. Quorum signaling, in potentially pathogenic bacteria, has been recognized as a necessary process by which biofilms regulate formation, structure, and detachment, although as yet, we have not discovered these pathways in the commensal microbiota. As there is so little knowledge of this unique, complex, and highly important system, this is an exciting field of study for those in microbiology, nutrition, and mathematical modeling.

This work is funded by the Riddet Institute. A.S.V.W. is supported by the Earle Food Fellowship.

The authors have declared no conflict of interest.

5 References

[1] Granato, D., Branco, G. F., Nazzaro, F., Cruz, A. G., Faria, J. A., Functional foods and nondairy probiotic food development: trends, concepts, and products. *Compr. Rev. Food Sci. Food Saf.* 2010, *9*, 292–302.

[2] Flegal, K. M., Carroll, M. D., Ogden, C. L., Curtin, L. R., Prevalence and trends in obesity among US adults, 1999–2008. *J. Am. Med. Assoc.* 2010, *303*, 235–241.

[3] Panico, S., Mattiello, A., Epidemiology of cardiovascular diseases in women in Europe. *Nutr. Metab. Cardiovasc. Dis.* 2010, *20*, 379–385.

[4] Ginter, E., Simko, V., Diabetes type 2 pandemic in 21st century. *Bratisl. Med. J.* 2010, *111*, 134–137.

[5] Tallon, M. J., *Probiotics Success Strategies in Food and Drinks*, Business Insights Ltd. London 2010.

[6] Younes, H., Coudray, C., Bellanger, J., Demigné, C. et al., Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. *Br. J. Nutr.* 2001, *86*, 479–485.

[7] Albrecht, T., Almond, J., Alfa, M., Alton, G. G. et al., in: Baron, S. (Ed.), *Medical Microbiology*, The University of Texas Medical Branch at Galveston, Galveston 1996.

[8] Macfarlane, G. T., Macfarlane, S., Models for intestinal fermentation: association between food components, delivery systems, bioavailability and functional interactions in the gut. *Curr. Opin. Biotechnol.* 2007, *18*, 156–162.

[9] Glancey, A., McBain, A. J., Gilbert, P., in: Gilbert, P., Allison, D., Brading, M., Pratten, J. et al. (Eds.), *Biofilms: Coming of Age*, The Biofilm Club, Manchester 2007, pp. 107–117.

[10] Conly, J. M., Stein, K., Worobetz, L., Rutledge-Harding, S., The contribution of vitamin K_2 (menaquinones) produced by the intestinal microflora to human nutritional requirements for vitamin K. *Am. J. Gastroenterol.* 1994, *89*, 915–923.

[11] Madhu, A. N., Giribhattanavar, P., Narayan, M. S., Prapulla, S. G., Probiotic lactic acid bacterium from kanjika as a potential source of vitamin B_{12}: evidence from LC-MS, immunological and microbiological techniques. *Biotechnol. Lett.* 2010, *32*, 503–506.

[12] Burgess, C. M., Smid, E. J., van Sinderen, D., Bacterial vitamin B2, B11 and B12 overproduction: an overview. *Int. J. Food Microbiol.* 2009, *133*, 1–7.

[13] Borruel, N., Carol, M., Casellas, F., Antolin, M. et al., Increased mucosal tumour necrosis factor α production in Crohn's disease can be downregulated ex vivo by probiotic bacteria. *Gut* 2002, *51*, 659–664.

[14] Furrie, E., Macfarlane, S., Kennedy, A., Cummings, J. H. et al., Synbiotic therapy (*Bifidobacterium longum*/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 2005, *54*, 242–249.

[15] Saulnier, D. M., Kolida, S., Gibson, G. R., Microbiology of the human intestinal tract and approaches for its dietary modulation. *Curr. Pharm. Des.* 2009, *15*, 1403–1414.

[16] Macfarlane, G. T., Blackett, K. L., Nakayama, T., Steed, H., Macfarlane, S., The gut microbiota in inflammatory bowel disease. *Curr. Pharm. Des.* 2009, *15*, 1528–1536.

[17] Lievin, V., Peiffer, I., Hudault, S., Rochat, F. et al., *Bifidobacterium* strains from resident infant human gastrointestinal microflora exert antimicrobial activity. *Gut* 2000, *47*, 646–652.

[18] Coconnier, M. H., Bernet, M. F., Kernéis, S., Chauvière, G. et al., Inhibition of adhesion of enteroinvasive pathogens to human intestinal Caco-2 cells by *Lactobacillus acidophilus* strain LB decreases bacterial invasion. *FEMS Microbiol. Lett.* 1993, *110*, 299–305.

[19] De Ruyter, P. G. G. A., Kuipers, O. P., De Vos, W. M., Controlled gene expression systems for *Lactococcus lactis* with the food-grade inducer nisin. *Appl. Environ. Microbiol.* 1996, *62*, 3662–3667.

[20] MacDonald, T. T., Monteleone, G., Immunity, inflammation, and allergy in the gut. *Science* 2005, *307*, 1920–1925.

[21] Luckey, T. D., Introduction to intestinal microecology. *Am. J. Clin. Nutr.* 1972, *25*, 1292–1294.

[22] Macfarlane, S., Dillon, J. F., Microbial biofilms in the human gastrointestinal tract. *J. Appl. Microbiol.* 2007, *102*, 1187–1196.

[23] Hooper, L. V., MacPherson, A. J., Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat. Rev. Immunol.* 2010, *10*, 159–169.

[24] Hooper, L. V., Midwedt, T., Gordon, J. I., How host–microbial interactions shape the nutrient environment of the mammalian intestine. *Annu. Rev. Nutr.* 2002, *22*, 283–307.

[25] O'Toole, P. W., Claesson, M. J., Gut microbiota: changes throughout the lifespan from infancy to elderly. *Int. Dairy J.* 2010, *20*, 281–291.

[26] Suau, A., Bonnet, R., Sutren, M., Godon, J.-J. et al., Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl. Environ. Microbiol.* 1999, *65*, 4799–4807.

[27] Kurokawa, K., Itoh, T., Kuwahara, T., Oshima, K. et al., Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. *DNA Res.* 2007, *14*, 169–181.

[28] Ellrott, K., Jaroszewski, L., Li, W., Wooley, J. C., Godzik, A., Expansion of the protein repertoire in newly explored environments: human gut microbiome specific protein families. *PLoS Comput. Biol.* 2010, *6*, e1000798.

[29] Costerton, J. W., Rozee, K. R., Cheng, K. J., Colonization of particulates, mucous, and intestinal tissue. *Prog. Food Nutr. Sci.* 1983, *7*, 91–105.

[30] Macfarlane, S., Macfarlane, G. T., Composition and metabolic activities of bacterial biofilms colonizing food residues in the human gut. *Appl. Environ. Microbiol.* 2006, *72*, 6204–6211.

[31] Macfarlane, S., McBain, A. J., Macfarlane, G. T., Consequences of biofilm and sessile growth in the large intestine. *Adv. Dent. Res.* 1997, *11*, 59–68.

[32] Probert, H. M., Gibson, G. R., Bacterial biofilms in the human gastrointestinal tract. *Curr. Issues Intest. Microbiol.* 2002, *3*, 23–27.

[33] Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P. E., Macfarlane, G. T., Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 1987, *28*, 1221–1227.

[34] Macfarlane, G. T., Gibson, G. R., Cummings, J. H., Comparison of fermentation reactions in different regions of the human colon. *J. Appl. Bacteriol.* 1992, *72*, 57–64.

[35] Leitch, E. C. M., Walker, A. W., Duncan, S. H., Holtrop, G., Flint, H. J., Selective colonization of insoluble substrates by human faecal bacteria. *Environ. Microbiol.* 2007, *9*, 667–679.

[36] Penders, J., Thijs, C., Vink, C., Stelma, F. F. et al., Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 2006, *118*, 511–521.

[37] Jiménez, E., Delgado, S., Fernández, L., García, N. et al., Assessment of the bacterial diversity of human colostrum and screening of *staphylococcal* and *enterococcal* populations for potential virulence factors. *Res. Microbiol.* 2008, *159*, 595–601.

[38] Perez, P. F., Doré, J., Leclerc, M., Levenez, F. et al., Bacterial imprinting of the neonatal immune system: lessons from maternal cells? *Pediatrics* 2007, *119*, e724–e732.

[39] Satokari, R., Grönroos, T., Laitinen, K., Salminen, S., Isolauri, E., *Bifidobacterium* and *Lactobacillus* DNA in the human placenta. *Lett. Appl. Microbiol.* 2009, *48*, 8–12.

[40] Jiménez, E., Fernández, L., Marín, M. L., Martín, R. et al., Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Curr. Microbiol.* 2005, *51*, 270–274.

[41] Jiménez, E., Marín, M. L., Martín, R., Odriozola, J. M. et al., Is meconium from healthy newborns actually sterile? *Res. Microbiol.* 2008, *159*, 187–193.

[42] Jacobs, D. M., Gaudier, E., van Duynhoven, J., Vaughan, E. E., Non-digestible food ingredients, colonic microbiota and the impact on gut health and immunity: a role for metabolomics. *Curr. Drug Metab.* 2009, *10*, 41–54.

[43] De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M. et al., Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl. Acad. Sci. USA* 2010, *107*, 14691–14696.

[44] Ley, R. E., Turnbaugh, P. J., Klein, S., Gordon, J. I., Microbial ecology: human gut microbes associated with obesity. *Nature* 2006, *444*, 1022–1023.

[45] Walker, A. W., Duncan, S. H., Harmsen, H. J. M., Holtrop, G. et al., The species composition of the human intestinal microbiota differs between particle-associated and liquid phase communities. *Environ. Microbiol.* 2008, *10*, 3275–3283.

[46] Mirande, C., Kadlecikova, E., Matulova, M., Capek, P. et al., Dietary fibre degradation and fermentation by two xylanolytic bacteria *Bacteroides xylanisolvens* XB1A^T and *Roseburia intestinalis* XB6B4 from the human intestine. *J. Appl. Microbiol.* 2010, *109*, 451–460.

[47] Macfarlane, G. T., Steed, H., Macfarlane, S., Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *J. Appl. Microbiol.* 2008, *104*, 305–344.

[48] Cummings, J. H., MacFarlane, G. T., Role of intestinal bacteria in nutrient metabolism. *Clin. Nutr.* 1997, *16*, 3–11.

[49] Marteau, P., Pochart, P., Dore, J., Bera-Maillet, C. et al., Comparative study of bacterial groups within the human cecal and fecal microbiota. *Appl. Environ. Microbiol.* 2001, *67*, 4939–4942.

[50] Gristina, A., Biomaterial-centered infection: microbial adhesion versus tissue integration. *Clin. Orthop. Relat. Res.* 2004, *427*, 4–12.

[51] Flemming, H. C., Leis, A., Strathmann, M., Leon-Morales, F., in: McBain, A., Allison, D., Pratten, J., Spratt, D. et al. (Eds.), *Biofilms: Persistence and Ubiquity*, The Biofilm Club, Manchester 2005, pp. 67–81.

[52] Boland, T., Latour, R. A., Sutzenberger, F. J., in: An, Y. H., Friedman, R. J. (Eds.), *Handbook of Bacterial Adhesion: Principles, Methods and Appllcations*, Humana Press, Inc., Totowa 2000, pp. 29–41.

[53] DunneJr, W. M., Bacterial adhesion: seen any good biofilms lately? *Clin. Microbiol. Rev.* 2002, *15*, 155–166.

[54] Neu, T. R., Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. *Microbiol. Rev.* 1996, *60*, 151–166.

[55] De Kerchove, A. J., Elimelech, M., Calcium and magnesium cations enhance the adhesion of motile and

nonmotile *Pseudomonas aeruginosa* on alginate films. *Langmuir* 2008, *24*, 3392–3399.

[56] Liu, Y., Strauss, J., Camesano, T. A., Thermodynamic investigation of *Staphylococcus epidermidis* interactions with protein-coated substrata. *Langmuir* 2007, *23*, 7134–7142.

[57] Jass, J., Emerton, M. E., Robinson, G. B., Simpson, H. A. R. W., in: Wimpenny, J., Handley, P., Gilbert, P., Lappin-Scott, H., Jones, M. (Eds.), *Biofilms: Community Interactions and Control*, BioLine, Cardiff 1997, pp. 55–62.

[58] Lentle, R. G., Janssen, P. W. M., Physical characteristics of digesta and their influence on flow and mixing in the mammalian intestine: a review. *J. Comp. Physiol. [B]* 2008, *178*, 673–690.

[59] Lentle, R. G., Janssen, P. W. M., Manipulating digestion with foods designed to change the physical characteristics of digesta. *Crit. Rev. Food Sci. Nutr.* 2010, *50*, 130–145.

[60] Lentle, R. G., Hemar, Y., Hall, C. E., Stafford, K. J., Periodic fluid extrusion and models of digesta mixing in the intestine of a herbivore, the common brushtail possum (*Trichosurus vulpecula*). *J. Comp. Physiol. [B]* 2005, *175*, 337–347.

[61] Goller, C. C., Romeo, T., in: Romeo, T. (Ed.), *Bacterial Biofilms*, Springer, Berlin 2008, pp. 37–66.

[62] Jenkinson, H. F., Nobbs, A. H., Jakubovics, N. S., in: McBain, A., Allison, D., Brading, M., Rickard, A. et al. (Eds.), *Biofilm Communities: Order from Chaos?* BioLine, Cardiff 2003, pp. 97–103.

[63] Cookson, A. L., Cooley, W. A., Woodward, M. J., The role of type 1 and curli fimbriae of shiga toxin-producing *Escherichia coli* in adherence to abiotic surfaces. *Int. J. Med. Microbiol.* 2002, *292*, 195–205.

[64] Cookson, A. L., Woodward, M. J., in: Wimpenny, J., Gilbert, P., Walker, J., Brading, M., Bayston, R. (Eds.), *Biofilms: The Good, The Bad and The Ugly*, BioLine, Cardiff 1999, pp. 73–80.

[65] Hicks, S., Frankel, G., Kaper, J. B., Dougan, G., Phillips, A. D., Role of intimin and bundle-forming pili in enteropathogenic *Escherichia coli* adhesion to pediatric intestinal tissue in vitro. *Infect. Immun.* 1998, *66*, 1570–1578.

[66] Shipman, J. A., Cho, K. H., Siegel, H. A., Salyers, A. A., Physiological characterization of SusG, an outer membrane protein essential for starch utilization by *Bacteroides thetaiotaomicron*. *J. Bacteriol.* 1999, *181*, 7206–7211.

[67] Shipman, J. A., Berleman, J. E., Salyers, A. A., Characterization of four outer membrane proteins involved in binding starch to the cell surface of *Bacteroides thetaiotaomicron*. *J. Bacteriol.* 2000, *182*, 5365–5372.

[68] Xu, J., Mahowald, M. A., Ley, R. E., Lozupone, C. A. et al., Evolution of symbiotic bacteria in the distal human intestine. *PLoS Biol.* 2007, *5*, e156.

[69] Crittenden, R., Laitila, A., Forssell, P., Matto, J. et al., Adhesion of *bifidobacteria* to granular starch and its implications in probiotic technologies. *Appl. Environ. Microbiol.* 2001, *67*, 3469–3475.

[70] Macfarlane, G. T., Cummings, J. H., in: Philips, S. F., Pemberton, J. H., Shorter, R. G. (Eds.), *The Large Intestine: Physiology, Pathophysiology, and Disease*, Raven Press, Ltd., New York 1991.

[71] Wada, J., Ando, T., Kiyohara, M., Ashida, H. et al., *Bifidobacterium bifidum* lacto-N-biosidase, a critical enzyme for the degradation of human milk oligosaccharides with a type 1 structure. *Appl. Environ. Microbiol.* 2008, *74*, 3996–4004.

[72] O'Keefe, S. J. D., Nutrition and colonic health: the critical role of the microbiota. *Curr. Opin. Gastroenterol.* 2008, *24*, 51–58.

[73] Jones, M. L., Chen, H., Ouyang, W., Metz, T., Prakash, S., Microencapsulated genetically engineered *Lactobacillus plantarum* 80 (pCBH1) for bile acid deconjugation and its implication in lowering cholesterol. *J. Biomed. Biotechnol.* 2004, *1*, 61–69.

[74] Begley, M., Hill, C., Gahan, C. G. M., Bile salt hydrolase activity in probiotics. *Appl. Environ. Microbiol.* 2006, *72*, 1729–1738.

[75] Go, P. M. N. Y. H., Van Dieijen-Visser, M. P., Davies, B. I., Lens, J., Brombacher, P. J., Microbial flora and bile acid metabolism in patients with an ileal reservoir. *Scand. J. Gastroenterol.* 1988, *23*, 229–236.

[76] Hamilton, J. P., Xie, G., Raufman, J.-P., Hogan, S. et al., Human cecal bile acids: concentration and spectrum. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2007, *293*, G256–G263.

[77] Eyssen, H., Role of the gut microflora in metabolism of lipids and sterols. *Proc. Nutr. Soc.* 1973, *32*, 59–63.

[78] Ridlon, J. M., Kang, D.-J., Hylemon, P. B., Bile salt biotransformations by human intestinal bacteria. *J. Lipid Res.* 2006, *47*, 241–259.

[79] Thomas, L. A., Veysey, M. J., French, G., Hylemon, P. B. et al., Bile acid metabolism by fresh human colonic contents: a comparison of caecal versus faecal samples. *Gut* 2001, *49*, 835–842.

[80] Missirlis, Y. F., Katsikogianni, M., Theoretical and experimental approaches of bacteria-biomaterial interactions. *Materialwiss. Werkst.* 2007, *38*, 983–994.

[81] Hermansson, M., The DLVO theory in microbial adhesion. *Colloids Surf. B* 1999, *14*, 105–119.

[82] Hori, K., Matsumoto, S., Bacterial adhesion: from mechanism to control. *Biochem. Eng. J.* 2010, *48*, 424–434.

[83] Long, G., Zhu, P., Shen, Y., Tong, M., Influence of extracellular polymeric substances (EPS) on deposition kinetics of bacteria. *Environ. Sci. Technol.* 2009, *43*, 2308–2314.

[84] Fletcher, M., Attachment of *Pseudomonas fluorescens* to glass and influence of electrolytes on bacterium–substratum separation distance. *J. Bacteriol.* 1988, *170*, 2027–2030.

[85] Goulter, R. M., Gentle, I. R., Dykes, G. A., Issues in determining factors influencing bacterial attachment: a review using the attachment of *Escherichia coli* to abiotic surfaces as an example. *Lett. Appl. Microbiol.* 2009, *49*, 1–7.

[86] Boks, N. P., Norde, W., van der Mei, H. C., Busscher, H. J., Forces involved in bacterial adhesion to hydrophilic and hydrophobic surfaces. *Microbiology* 2008, *154*, 3122–3133.

[87] Pashley, R. M., McGuiggan, P. M., Ninham, B. W., Evans, D. F., Attractive forces between uncharged hydrophobic surfaces: direct measurements in aqueous solution. *Science* 1985, *229*, 1088–1089.

[88] Katsikogianni, M., Missirlis, Y. F., Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria–material interactions. *Eur. Cell Mater.* 2004, *8*, 37–57.

[89] Van Oss, C. J., Good, R. J., Chaudhury, M. K., The role of van der Waals forces and hydrogen bonds in ''hydrophobic interactions'' between bipolymers and low energy surfaces. *J. Colloid Interface Sci.* 1986, *111*, 378–390.

[90] Van Oss, C. J., Chaudhury, M. K., Good, R. J., Interfacial Lifshitz–van der Waals and polar interactions in macroscopic systems. *Chem. Rev.* 1988, *88*, 927–941.

[91] Van Oss, C. J., Energetics of cell–cell and cell–biopolymer interactions. *Cell Biochem. Biophys.* 1989, *14*, 1–16.

[92] Schneider, R. P., Conditioning film-induced modification of substratum physicochemistry – analysis by contact angles. *J. Colloid Interface Sci.* 1996, *182*, 204–213.

[93] Bayoudh, S., Othmane, A., Mora, L., Ben Ouada, H., Assessing bacterial adhesion using DLVO and XDLVO theories and the jet impingement technique. *Colloids Surf. B* 2009, *73*, 1–9.

[94] Hori, K., Matsumoto, S., Bacterial adhesion: from mechanism to control. *Biochem. Eng. J.* 2009, *48*, 424–434.

[95] Liu, X., Lim, J. Y., Donahue, H. J., Dhurjati, R. et al., Influence of substratum surface chemistry/energy and topography on the human fetal osteoblastic cell line hFOB 1.19: phenotypic and genotypic responses observed in vitro. *Biomaterials* 2007, *28*, 4535–4550.

[96] Costerton, J. W., Cheng, K. J., Geesey, G. G., Ladd, T. I. et al., Bacterial biofilms in nature and disease. *Annu. Rev. Microbiol.* 1987, *41*, 435–464.

[97] Bayston, R., in: Wimpenny, J., Gilbert, P., Walker, J., Brading, M., Bayston, R. (Eds.), *Biofilms: The Good, The Bad and The Ugly*, BioLine, Cardiff 1999, pp. 1–6.

[98] Sandiford, S., Tagg, J., Upton, M., in: Gilbert, P., Allison, D., Brading, M., Pratten, J. et al. (Eds.), *Biofilms: Coming of Age*, The Biofilm Club, Manchester 2007, pp. 145–156.

[99] Hasty, D. L., Ofek, I., Courtney, H. S., Doyle, R. J., Multiple adhesins of *Streptococci*. *Infect. Immun.* 1992, *60*, 2147–2152.

[100] Koo, H., Xiao, J., Klein, M. I., Jeon, J. G., Exopolysaccharides produced by *Streptococcus mutans* glucosyltransferases modulate the establishment of microcolonies within multispecies biofilms. *J. Bacteriol.* 2010, *192*, 3024–3032.

[101] Vu, B., Chen, M., Crawford, R. J., Ivanova, E. P., Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules* 2009, *14*, 2535–2554.

[102] Sutherland, I. W., Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology* 2001, *147*, 3–9.

[103] Marvasi, M., Visscher, P. T., Casillas Martinez, L., Exopolymeric substances (EPS) from *Bacillus subtilis*: polymers and genes encoding their synthesis. *FEMS Microbiol. Lett.* 2010, *313*, 1–9.

[104] Donlan, R. M., Biofilms: microbial life on surfaces. *Emerg. Infect. Dis.* 2002, *8*, 881–890.

[105] Sutherland, I. W., The biofilm matrix – an immobilized but dynamic microbial environment. *Trends Microbiol.* 2001, *9*, 222–227.

[106] Wanner, O., Eberl, H. J., Morgenroth, E., Noguera, D. I. R. et al., *Mathematical Modeling of Biofilms*, IWA Publishing, London 2006.

[107] Anderl, J. N., Zahller, J., Roe, F., Stewart, P. S., Role of nutrient limitation and stationary-phase existence in *Klebsiella pneumoniae* biofilm resistance to ampicillin and ciprofloxacin. *Antimicrob. Agents Chemother.* 2003, *47*, 1251–1256.

[108] Hammer, B. K., Bassler, B. L., Quorum sensing controls biofilm formation in *Vibrio cholerae*. *Mol. Microbiol.* 2003, *50*, 101–104.

[109] Rhoads, D. D., Wolcott, R. W., Cutting, K. F., Percival, S. L., in: Gilbert, P., Allison, D., Brading, M., Pratten, J. et al. (Eds.), *Biofilms: Coming of Age*, The Biofilm Club, Manchester 2007, pp. 131–143.

[110] Zhu, J., Mekalanos, J. J., Quorum sensing-dependent biofilms enhance colonization in *Vibrio cholerae*. *Dev. Cell* 2003, *5*, 647–656.

[111] Davies, D. G., Parsek, M. R., Pearson, J. P., Iglewski, B. H. et al., The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 1998, *280*, 295–298.

[112] Barken, K. B., Pamp, S. J., Yang, L., Gjermansen, M. et al., Roles of type IV pili, flagellum-mediated motility and extracellular DNA in the formation of mature multicellular structures in *Pseudomonas aeruginosa* biofilms. *Environ. Microbiol.* 2008, *10*, 2331–2343.

[113] Davey, M. E., Caiazza, N. C., O'Toole, G. A., Rhamnolipid surfactant production affects biofilm architecture in *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.* 2003, *185*, 1027–1036.

[114] Boles, B. R., Thoendel, M., Singh, P. K., Rhamnolipids mediate detachment of *Pseudomonas aeruginosa* from biofilms. *Mol. Microbiol.* 2005, *57*, 1210–1223.

[115] Danese, P. N., Pratt, L. A., Kolter, R., Exopolysaccharide production is required for development of *Escherichia coli* K-12 biofilm architecture. *J. Bacteriol.* 2000, *182*, 3593–3596.

[116] O'May, G. A., Reynolds, N., Smith, A. R., Kennedy, A., Macfarlane, G. T., Effect of pH and antibiotics on microbial overgrowth in the stomachs and duodena of patients undergoing percutaneous endoscopic gastrostomy feeding. *J. Clin. Microbiol.* 2005, *43*, 3059–3065.

[117] Guarner, F., Malagelada, J. R., Gut flora in health and disease. *Lancet* 2003, *361*, 512–519.