

Bacterial Biofilms In The Human Gastrointestinal Tract

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Abstract

Microbial biofilms were first described in 1936 and subsequent research has unveiled their ubiquity and physiological distinction from free-living (planktonic) microorganisms. In light of their emerging significance this review examines the bacterial biofilms within the human gastrointestinal tract. Attention is paid to the nature of these mucosally-associated populations, focusing on the protected environment afforded by the continual secretion of mucus by host epithelial cells. It also examines the attributes possessed by various bacterial species that facilitate habitation of this microenvironment. Additionally, contrasts are drawn between planktonic bacteria of the lumen and sessile (biofilm) bacteria growing in close association with host cells and food particles. In particular the different fermentation profiles exhibited by these two fractions are discussed. The potential role of these communities in host health and disease, as well as the stabilisation of the luminal population, is also considered. Reference is made to the state of mutualism that exists between these little understood populations and the host epithelia, thus highlighting their ecological significance in terms of gastrointestinal health.

Introduction

The first description of bacterial biofilms (Zobell and Andersen, 1936) marked the start of an ever increasing understanding of the significance of these microbial communities. Current research continues to extend our knowledge and understanding in a previously underestimated field. The stereotypical idea of free-living or planktonic bacteria suspended in a liquid growth medium is typically a laboratory concept and, in reality, this mode of microbial life only represents a small proportion of how bacteria actually exist in their ecosystems. Bacteria show a propensity to grow in communities in association with a surface in preference to individual planktonic growth.

In microbiological terms, a 'biofilm' describes matrix-enclosed bacterial populations adherent to each other and/or surfaces or interfaces. This includes microbial aggregates and flocules (Costerton *et al.*, 1995). Biofilms are ubiquitous and are found in a huge range of natural environments. One such habitat in need of more extensive

research is the human gastrointestinal tract, where the significance of mucosally-associated bacteria still needs to be evaluated.

The Human Colonic Microbiota

A huge variety of bacterial species inhabit the human large intestine, constituting an extremely complex ecosystem and rendering it a site of intense metabolic activity. Approximately 150 cm long and containing in the region of 220g of contents (Cummings and Macfarlane, 1991), it has been proposed that within the large intestine at least 400 – 500 different culturable species of bacteria reside (Conway, 1995), each fulfilling a specialised ecological niche. In reality, it is likely that many more species than this exist, since the full gut flora diversity has not hitherto been described.

The human colon is sterile at birth, with microbial inoculation occurring during parturition (Macfarlane and McBain, 1999). Generally, the first colonisers are facultative anaerobes *e.g.* enterococci and enterobacteria, followed by obligate anaerobes. Differences have been highlighted between breast fed and formula fed infants with the former giving rise to a flora largely dominated by bifidobacteria whilst formula fed infants have a more complex, adult like microbiota, with no one genus showing any overall predominance (Conway, 1995). Upon weaning, these differences disappear and a complex adult type flora becomes established (Macfarlane *et al.*, 1999).

In terms of the planktonic, luminal populations, numerically dominant species in this complex adult microbiota are non-spore forming anaerobes belonging to the genera *Bacteroides*, *Eubacterium* and *Bifidobacterium*. To a lesser extent, lactobacilli, a variety of anaerobic Gram-positive cocci and clostridia are also present (Cummings *et al.*, 1991). These bacteria, and many more, constitute a microbial ecosystem in the magnitude of 10^{13} cells, which accounts for the vast majority of total cells associated with the human body (Macfarlane *et al.*, 1999).

To date, most information on the colonic microbiota has been generated from studies of the planktonic bacteria found in faeces. However, it is the case that sessile bacteria forming biofilms in the mucus layer of the gut are likely to play a pivotal role in gut health and disease (Croucher *et al.*, 1983).

The Mucosal Habitat

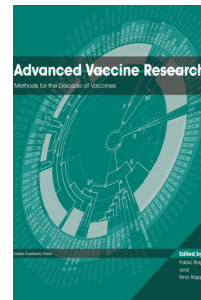
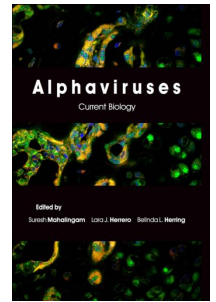
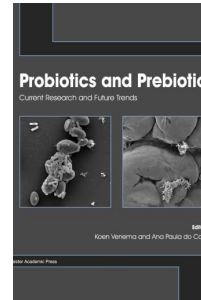
From the stomach to the colon the gastrointestinal tract is covered with a layer of mucus which forms an interface between the host and the gut lumen (Atuma *et al.*, 2001). Indeed, this mucus, adherent to the surface of the epithelial cells, forms a protective gel such that in the normal healthy situation the host cells are never directly exposed to luminal contents (Pullan *et al.*, 1994). The mucus layer is produced by goblet cells that reside in the intestinal

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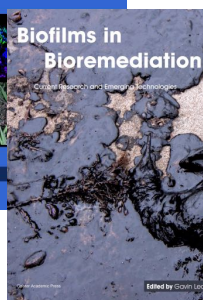
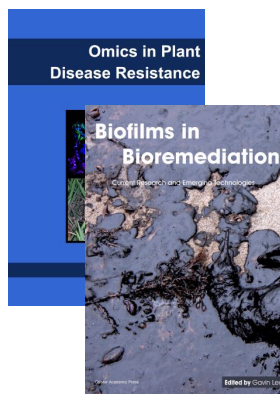
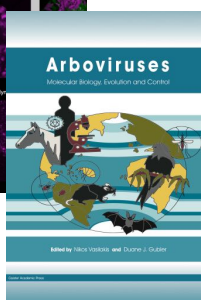
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epithelium, which are able to synthesise and secrete mucins which are responsible for the characteristic gel-like properties of this layer (Clamp *et al.*, 1981). Investigations by Pullan *et al.* (1994) found that this continuous layer varied in thickness between healthy samples, showing a general increase from the right colon to the rectum. Indeed, mucus thickness in the group studied varied from 48 to 273 μm .

As well as its generally accepted protective function, this layer also acts as a lubricant for the transit of luminal contents along the digestive tract (Atuma *et al.*, 2000). More importantly, from a microbiological viewpoint, this protected, undisturbed layer creates a microenvironment for the autochthonous microflora. A state of equilibrium exists between the growth of bacteria in the mucus gel and shedding of mucus (and epithelial cells) into the lumen. Mucus is constantly shed into the lumen and gut epithelial cells have a turnover time of 3-6 days (McNeil and Ito, 1989; Poulsen *et al.*, 1995). The persistence of an organism in this environment depends on its ability to replicate faster than it can be shed into the lumen and expelled from the body (Cohen *et al.*, 1985).

Studies carried out hitherto suggest that some bacteria adhere to the colonic mucus whereas others do not. Furthermore, it would appear that certain microorganisms are attached to the epithelium and extend from this into the mucus blanket covering the cells (Roze *et al.*, 1982). Studies indicate that sessile bacteria are mostly associated with the epithelial cell surface (Davis, 1976) whilst other investigations suggest that the reverse is true and that bacteria are more concentrated in the mucus layer away from the host epithelium (Croucher *et al.*, 1983).

Not only can bacteria exist as biofilms on the colonic epithelium and within the mucus layer covering it but they can also form associations with food particles in the lumen. Macfarlane *et al.*, (1997) found that approximately 5% of bacterial mass in luminal contents could be found in close association with food particles. Moreover, a much higher percentage is more loosely associated with particulate matter.

In the case of the indigenous, benign mucosal microflora Poulsen *et al.*, (1995) found that a strain of *Escherichia coli* (strain BJ4) appeared to exist in two distinct populations. One population in the mucus layer had a generation time of 40-80 minutes, whereas the luminal population (derived from sloughed off mucosal populations) was virtually static and contributed little to growth. Whether this is true of any other bacterial populations has yet to be investigated but it would appear that the luminal flora comprises many bacterial cells that have been sloughed off from mucosal populations.

Predominant Bacteria

Mucosally-associated bacterial populations in the colon are inherently difficult to study since invasive methods must be employed in order to obtain samples for analysis. Consequently, whilst much has been learnt about the human faecal flora, relatively little data are available on mucosally-associated bacteria in the gut (Poxton, 1997). Compounding this is the fact that the samples obtained

are often, by nature of the procedure involved, derived from diseased individuals, and not healthy subjects. Analyses of such samples may not therefore give an accurate depiction of the *in vivo* situation in the normal healthy human (Edmiston *et al.*, 1982).

Advances in electron microscopy techniques have enabled mucosal populations to be viewed without disruption to the mucus layer covering the epithelium (Roze *et al.*, 1982). This has supplied valuable information on the nature of colonisation of the epithelia and mucus by bacteria. Scanning electron microscopy (SEM) studies of the colonic mucosa has enabled the qualitative visualisation of bacterial species that exist in association with this interface between the lumen and the host (Bayliss and Turner, 1982). Furthermore, resuspension and culturing of these bacteria has provided an indication of the species involved and their relative numbers (Croucher *et al.*, 1983).

The predominant anaerobes found to be associated with mucosal tissue are *Bacteroides* and *Fusobacterium* spp. Indeed, fusobacteria have been found to have a "bridging" function within biofilms, forming coaggregation/cohesion bridges between early colonisers and late colonisers and thus contributing towards biofilm establishment and accumulation (Kolenbrander *et al.*, 2000). This may also explain why only very low numbers of fusobacteria are isolated from faeces since their role in colonic ecology may well be predominantly mucosally-associated. Indeed, fusobacteria appear to have a preference for adopting sessile growth. Extensive studies of dental plaque biofilm communities have similarly highlighted the pivotal role of these bacteria in biofilm formation and perpetuation. It has been shown that fusobacteria are capable of co-aggregating with every species of oral bacteria tested so far, as well as *Helicobacter pylori*, which actually selectively binds to *Fusobacterium* spp. (Andersen *et al.*, 1998; Kolenbrander *et al.*, 2000).

The permissiveness of fusobacteria allows these anaerobic bacteria to persist in aerated environments such as the mouth. Indeed, experiments have shown that oral anaerobes, such as fusobacteria, cannot survive without interacting with facultative or aerobic species within a biofilm. Furthermore, the favourable ecological niche supplied by the film is not afforded in communities that are lacking in oxygen consuming species (Bradshaw, 1997).

A similar situation possibly exists in the gastrointestinal tract where fusobacteria may interact with facultative species such as *Escherichia coli* at the mucosal surface. Perhaps the role of the aerobes in this location is to mop up any oxygen that diffuses from the host blood stream into the lumen rendering the environment more favourable for fastidious anaerobes (Savage, 1986). Such facultative bacteria act as oxygen scavengers and could possibly interact and co-aggregate with fusobacteria to form mucosal biofilms which remove traces of oxygen and allow anaerobes to flourish. Fusobacteria are also known to form complex interactions with spirochaetes, shown to be present in mucosal biopsy sections. Interestingly, fusobacterial numbers in faeces are often low but can be completely absent from a sample, which led to early deductions that they were not indigenous in the normal

Table 1. Bacterial groups associated with the luminal and mucosal environment of the human colon. It must be borne in mind that these are just some of the bacteria commonly associated with these regions and that the full diversity of the gut microflora has not yet been described.

Luminal/planktonic bacteria	Mucosally-associated/biofilm bacteria
<i>Bacteroides</i>	<i>Bacteroides</i>
<i>Eubacterium</i>	<i>Fusobacterium</i>
<i>Bifidobacterium</i>	Spirochaetes
<i>Lactobacillus</i>	<i>Escherichia coli</i>
Gram positive cocci	Helicobacter
<i>Clostridium</i>	<i>Bifidobacterium</i>
	Gram positive cocci

faecal flora (Van Houte and Gibbons, 1966). However, studies of mucosal populations have revealed that this microorganism is prevalent in biofilm communities.

It seems likely that certain bacteria have a competitive advantage when colonising the mucus layer. For example, spirochetes have a unique structure which results in a motility that enables them to swim in highly viscous, gel-like media. Hence, such bacteria can successfully penetrate the colonic mucus and reside therein (Li *et al.*, 2000). Other major types of bacteria found in this region include *Bifidobacterium* spp. and anaerobic Gram-positive cocci (Croucher *et al.*, 1983). A comparison between luminal and mucosal bacterial populations can be found in Table 1.

The mucus layer can also act as a source of nutrients to intestinal bacteria, such as bacteroides and bifidobacterial species (Hoskins, 1985). In terms of fermentation, this flora is better adapted to utilise long chain endogenous substrates, such as mucin, when compared to planktonic counterparts. Conversely, luminal bacteria are better equipped to ferment the shorter chain exogenous dietary substrates (Macfarlane *et al.*, 1997). It is not surprising, therefore, that there is a very high degree of stability with respect to attached mucosal bacteria in the intestine (Edmiston *et al.*, 1982), since such sessile bacteria are little affected by the exogenous influences of the diet, and seemingly work to homeostatically regulate luminal populations.

Studying the fermentation profile of bacteria growing in association with food particles has revealed that activities of arabinogalactanase and xylanase were increased in microorganisms growing in association with food particles compared to those growing entirely planktonically in the lumen. Conversely, pectinase and amylase levels were comparable. These particle-associated bacteria also expressed higher levels of α -galacturonidase, β -xylosidase and β -galacturonidase. On the other hand, relatively low levels of β -glucuronidase were present (Macfarlane *et al.*, 1997). Such results, in conjunction with other studies, strongly suggest that bacteria growing in association with food particles, *i.e.* in a biofilm, possess a different metabolic profile to their planktonic counterparts. Interestingly, these particle-associated bacteria were unable to metabolise mucin as efficiently as the planktonic bacteria, which in turn cannot utilise this substrate as efficiently as mucosal populations.

Although this hardly compares to the relative wealth of information on the luminal microflora it has begun to

shape our further understanding of the nature of the biofilms existing in the colon and hence their possible influences on health (and disease) of the host. Additionally, such findings seem to suggest that these previously overlooked populations may play a significant role in stabilising the luminal flora.

Role Of Mucosal Populations In Health And Disease

Unsurprisingly, microorganisms straddling the boundary between the host and the lumen can play a pivotal role in the health status of an individual. Indeed, a state of mutualism exists between bacteria residing in this mucus layer and host colonocytes beneath. This mutualism arises from the fact that short chain fatty acids produced in fermentation by this microflora can be utilised as an energy source by host cells whilst the bacteria simultaneously use the mucosal cells and their mucus secretions as nutrients (Marsh and Bowden, 2000). Furthermore, the benign autochthonous microflora can confer a form of colonisation resistance against potential pathogens, rendering it more difficult for opportunistic organisms to penetrate the colonic mucosa and elicit an infection (Isolauri *et al.*, 1999). Indeed, the barrier effect of these organisms is considerable in the colon, where bacterial colonisation is high, whereas in more proximal areas of the gastrointestinal tract potential pathogens have to overcome a much weaker barrier since bacterial colonisation is far less dense.

This can clearly be seen in the case of pathogens such as enterotoxigenic *Escherichia coli* which targets the small intestine where the barrier effect of the autochthonous microflora is low due to higher acidity and peristaltic movements in this region (Macfarlane and Macfarlane, 1997). This organism adheres to and colonises the mucus in order to elicit a pathogenic effect (Knutton *et al.*, 1984). This means that the pathogen and/or its toxins can readily adhere to exposed colonocytes and invade the host (Schiffirin and Brassart, 1995). It is in this respect that the normal mucosal microflora of the colon can confer a positive effect on host health.

In terms of pathogens, the ability to penetrate the mucus is often seen as an integral part of successful invasion but penetration of the colonic mucus may be unfavourable if the invading organism is sloughed off with the mucus faster than it is able to replicate and gain a strong foothold. In this way, mucus is a vital host defence against opportunistic pathogens, its efficacy depending on the bacterium's ability to persist. In essence, it would seem that an organism's ability to adhere to a surface determines the level of success.

The discovery of certain bacteria in this region has been coupled with particular disease states. Spirochaetes have previously been associated with a spectrum of diseases ranging from ulcerative colitis to rheumatoid arthritis (Lee *et al.*, 1971). However, since their appearance in healthy subjects, doubt has been cast on their aetiological significance. On the other hand, another spiral organism, *Helicobacter pylori*, which is found in the human gastric mucosa has a far more defined aetiological significance, since this agent is believed to be a co-factor in the development of gastric ulcers and other related

diseases, including stomach carcinomas and Type B gastritis (Jalava *et al.*, 1998). The pathogen is able to colonise the gastric epithelial cell surface of the stomach with apparent target cell specificity (Kobayashi *et al.*, 1993). In addition to its attachment to host cell surfaces, it can be found within and beneath the mucus layer (Clyne and Drumm, 1993).

Attempts have been made to identify possible differences between bacteriological profiles of the mucosally-associated populations in diseased and healthy individuals. Notably, conditions such as ulcerative colitis have, unsurprisingly, warranted research into the colonic mucosal microflora whereby differences have been sought between the microflora in healthy individuals and those with this, and other, chronic conditions. The mucosal microflora has been implicated in this form of inflammatory bowel disease due to the high recovery rate of L-forms, otherwise known as cell-wall deficient organisms or spheroplasts, in patients with ulcerative colitis. The L-forms are resistant to the host defences since they lack cell wall antigens, thus facilitating their intracellular survival (Chadwick and Anderson, 1995). The thickness of this mucus layer has aetiological significance in terms of inflammatory diseases. The mucus layer is greatly diminished or completely absent in areas of acute inflammation in the case of ulcerative colitis whereas the mucus thickness in samples from patients with Crohn's disease were the same as the control samples or thicker (Pullan *et al.*, 1994; McCormick *et al.*, 1990).

It can be seen, therefore, that the ability of bacteria to attach to the host has an enormous impact on the microbial ecology of the large intestine which subsequently confers both advantageous and detrimental effects to the individual.

Concluding Remarks

Since the existence of biofilms was discovered our knowledge and understanding of these bacterial communities has increased markedly. Microbiology is now at a stage where it is recognised that rather than biofilm growth being one small facet of an otherwise planktonic existence, the reverse is in fact true – and free-living bacteria may only represent a small piece of the overall picture. Inherently more difficult to study than their planktonic counterparts, sessile bacterial cells pose a variety of challenges to the researcher. This could not be more relevant than in the case of the biofilms in the human gastrointestinal tract, where relatively speaking our current knowledge is scant. However, it is likely that these populations exert a much more marked effect on host health than lumenal populations.

Tissue culture techniques using human epithelial cells enables us to model, to some extent, bacterial adhesion *in vitro*. This has been used to model the attachment of potentially harmful bacteria such as *Escherichia coli* (Winsor *et al.*, 1992), *Clostridium difficile* (Naaber *et al.*, 1996) and *Bacteroides fragilis* (Brook and Myhal, 1991) to host cells. Conversely, the adhesion of innocuous, or even potentially beneficial members of the colonic microflora has been examined, such as lactobacilli (Elo *et al.*, 1991) and bifidobacteria (Bernet *et al.*, 1993). What these studies

cannot do, however, is begin to model the complexity of the attached colonic microflora where many hundreds of bacterial species interact to form such a diverse and intricate ecosystem. Investigations would be useful where the adhesion of greater numbers of bacterial species are examined concomitantly. Such an investigation would help give an insight into the colonisation of the gastrointestinal tract following parturition.

New designs in continuous culture equipment would mean that biofilms could be cultured over longer periods of time and hence more could be gleaned about these bacterial communities. Specifically, the effect of exogenous dietary substrates on the attached colonic microflora could be examined as well as the influence of antibiotics on such populations.

The cultivation of biofilms via tissue culture techniques and novel fermentation equipment could pave the way to further our knowledge in this challenging area of microbiology, and hence better comprehend their influence on host health and disease.

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