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REVIEW ARTICLE

Microbial biofilms in the human gastrointestinal tract

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Summary

The human gastrointestinal tract contains rich and diverse microbiotas along its length. However, while extensive studies have been made on lumenal bacterial communities in the gut, less work has been carried out on organisms growing in biofilms, where individual groups of bacteria exist in a multiplicity of different microhabitats and metabolic niches associated with the mucosa, the mucus layer and particulate surfaces in the gut lumen. Bacteria and yeasts also occur in biofilms attached to artificial surfaces and devices implanted in the host, such as in patients being fed via enteral tubes. Although we are just beginning to investigate the composition and metabolic activities of these structures, increasing evidence suggests that they are important to the host in both health and disease. There is mounting interest in mucosal biofilms in the colon, especially with respect to their role in inflammatory bowel disease. Because bacteria growing in biofilms are more resistant to antibiotics than unattached organisms, it is often difficult to modify the structure and composition of these communities, or to eradicate them from the body. However, recent work has shown that there is considerable potential to alter the species composition of mucosal biofilms in a beneficial way using synbiotics.

Introduction

Mucosal bacterial communities in the upper gastrointestinal (GI) tract and large bowel are difficult to study in healthy people, and until relatively recently, this has limited investigations on their composition, structure and function. Biopsies and surgical material are usually obtained from diseased individuals, or from patients who have received antibiotic therapy, and from those whose colons have been washed-out before endoscopy or colonoscopy. As a consequence, microbial communities found attached to these tissues may not provide a true indication of normal mucosal diversity. Despite problems with sample acquisition, there is a body of evidence indicating the existence of independent mucosal communities in the human digestive tract (Lee *et al.* 1971; Croucher *et al.* 1983; Macfarlane *et al.* 2004).

In the initial stages of colonization or infection of mucosal surfaces in the digestive tract, micro-organisms need to be able to withstand shear forces resulting from the flow of material in the gut lumen, to avoid being physically removed from epithelial surfaces (Smith 1995). Epithelial cells lining the gut are covered by a layer of mucus, which evidence suggests may prevent the majority of organisms from reaching the epithelial surface (Florey 1955). Mucus forms a viscoelastic gel (Allen 1981), and it is its gel-like properties that are generally protective against adhesion and invasion by pathogenic micro-organisms, microbial toxins and other toxic waste products of bacterial metabolism, pancreatic endopeptidases and other hydrolases, foreign antigens and other damaging agents that occur in the gut lumen.

The large intestine is the site most heavily colonized by micro-organisms in the GI tract. While the colonic microbiota is usually thought of as being a homogeneous entity, this is an over simplification, because the bacteria exist in a multiplicity of different microhabitats and metabolic niches in the mucus layer lining the gut, the mucosa and on the surfaces of digestive residues in the gut lumen. These microcosms are continually changing, as nutrients are consumed or new resources become available. Microscopic evidence shows that bacteria in the large intestine can occur independently as individual cells, but many exist in microcolonies, or in disparate

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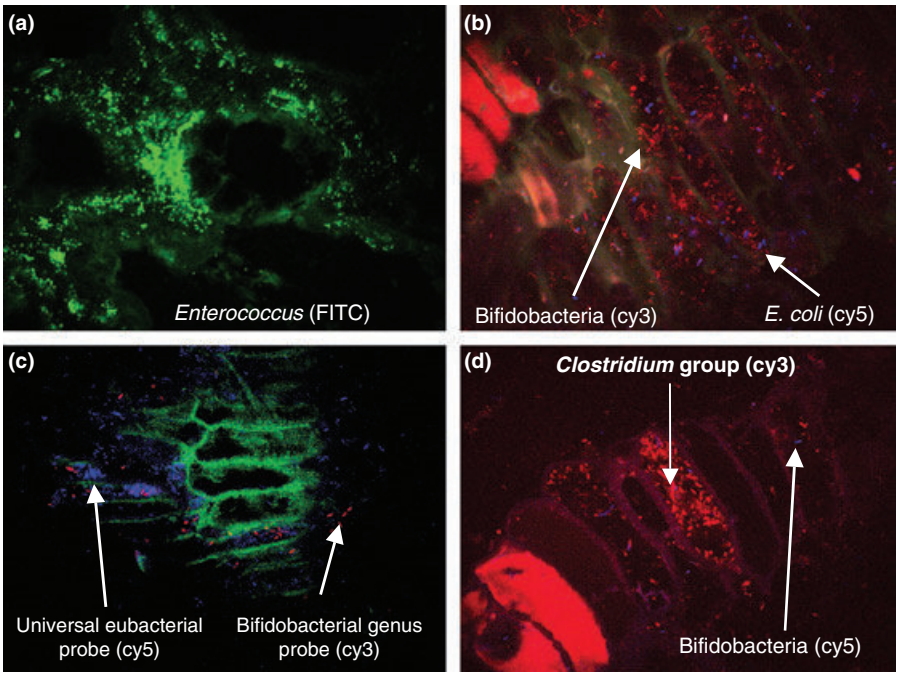

Figure 1 FISH light micrographs showing bacterial biofilms on the colonic mucosae of a patient with ulcerative colitis (a) and plant tissues extracted from faecal material (b–d). The samples were hybridized with a range of fluorescent genus and species-specific 16S rRNA oligonucleotide probes.

associations with other species on the surfaces of particulate materials (Fig. 1). In some circumstances, microbial biofilms can consist of a single species, as in infections of heart valves, catheters and medical prosthetic devices, but biofilms associated with different regions of the GI tract are usually multispecies consortia whose development is determined by environmental and nutritional factors, as well as by the chemical composition of the substratum and host defensive mechanisms associated with the innate and adaptive immune systems.

Microbial biofilms are widespread in nature, and they exist in a variety of environments including soils and sediments, as well as the skin, mouth and gut in humans. While much is known about the composition, structure and metabolism of oral biofilms, their study has largely been neglected in the GI tract. However, based on what we know of biofilms in the oral cavity, it is likely that particle-associated and mucosal biofilm communities in the lower digestive tract, particularly the large bowel are highly evolved assemblages. Biofilm communities often exhibit coordinated multicellular behaviour, within and between species, and many biofilm properties are dependent on local cell population densities. A good example of this is provided by quorum sensing transcriptional activation in certain Gram-negative bacteria (Salmond *et al.* 1995). Close spatial relationships between bacterial cells growing on surfaces are important in other ways, particularly in relation to metabolic communication between micro-organisms and the potentially growth-limiting effects associated with mass transfer resistance (Macfarlane *et al.* 2000).

Micro-organisms growing in biofilms frequently express phenotypes that are different from their nonadherent counterparts. For example, the nature and efficiency of their metabolism is changed, while many species in the biofilm exhibit greater resistance to antibiotics and other deleterious environmental factors, such as acid pH and host defensive measures that are inhibitory to free-living organisms (Anwar *et al.* 1990; van Loosdrecht *et al.* 1990; Mozes and Rouxhet 1992).

The upper gastrointestinal tract

Normal oesophageal microbiota

In quantitative terms, the oesophagus and stomach carry the lightest microbial loads in the human digestive tract. In healthy individuals the normal oesophageal microbiota has generally received little attention from microbiologists; however, studies have shown that it is relatively simple in terms of species composition, and that the predominant culturable bacteria are facultative anaerobes originating in the oral cavity, such as streptococci and lactobacilli, which occur in relatively low

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numbers (*c.* 10^2–10^3 cm^{-2} or ml^{-1} of the mucosal surface or lumenal aspirate, respectively).

Bacterial populations in patients with Barrett's oesophagus

Barrett's oesophagus is the term applied to a metaplasia of the lower end of the oesophagus in which the usual squamous mucosa has transformed into a columnar lined mucosa. Barrett's oesophagus arises in patients with gastro-oesophageal reflux disease, where approximately 10% develop Barrett's. People with Barrett's oesophagus have a greatly increased risk of developing oesophageal adenocarcinoma. This form of cancer has undergone a rapid rise in incidence over the last 20 years, and is now the seventh commonest cause of cancer death in the United Kingdom. It is believed that all adenocarcinomas arise in Barrett's, but only 1 in 20 patients has a previous diagnosis of the condition. Treatment for adenocarcinoma of the oesophagus is almost invariably unsuccessful, with 5 year survival rates as low as 5%. Neither aggressive drug therapies nor antireflux surgical procedures prevent Barrett's or its progression to cancer. There must be an environmental or life style factor to account for the rapid change in incidence for both Barrett's and adenocarcinoma of the oesophagus over the last two decades.

In view of the well-known role of *Helicobacter pylori* in the aetiology of gastric cancer, it may be instructive to consider the activities of bacteria comprising the microbiota of the upper GI tract. *Helicobacter pylori* has been shown conclusively to cause duodenal ulcer disease as well as gastric cancer, by indirect mechanisms, because of chronic colonization of the superficial gastric mucosa. This bacterium has been studied in Barrett's oesophagus, and after much controversy, the organism has been shown to have no role in its development. Studies have shown that there are no *H. pylori* growing within the Barrett's mucosa, despite the fact that it is a gastric-type mucosa. If *H. pylori* is not linked to the disease, is an abnormal microbiota associated with the transformation of Barrett's mucosa to adenocarcinoma? We have found that there is greater species diversity and higher numbers of bacteria in the distal oesophagus in Barrett's patients, compared with the situation in healthy people, and that there can be extensive microcolony formation on the epithelial surface (Figs 2 and 3). Many of these species are nitrate reducers, and may ultimately be responsible for DNA damage through the production of *N*-nitroso compounds, substances that have long been implicated as carcinogens in the lower oesophagus and stomach. Nitrite produced from salivary nitrate can also react with gastric acid to generate nitric oxide, a highly diffusible molecule,

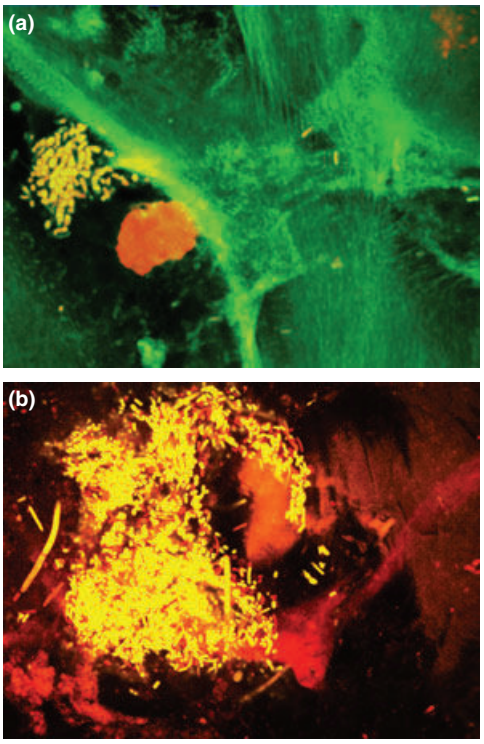

Figure 2 Live/dead stain of healthy oesophageal tissue showing sparse growth and the presence of a small microcolony (a), and tissue taken from a patient with Barrett's oesophagus showing bacterial growth in aggregates (b). Yellow cells are living, red bacteria are dead.

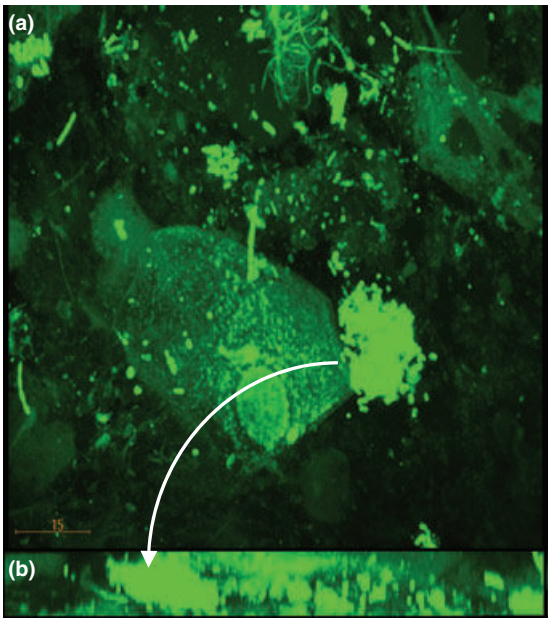

Figure 3 Live/dead stain of a section of tissue taken from a patient with Barrett's oesophagus using laser scanning confocal microscopy showing rods and cocci on the epithelial surface (a) and a vertical section of the mucus layer (b). Green staining bacteria are living.

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which has been shown to be mutagenic in high doses. The production of nitric oxide occurs maximally at the gastro-oesophageal junction, where it is able to diffuse rapidly into the surrounding epithelial cells and potentially contribute to the high incidence of neoplasia at that site.

Bacterial colonization of the stomach and upper small bowel

Except for a few hours immediately following a meal, the human stomach is largely devoid of a significant microbiota in healthy people, containing only relatively low numbers of lactobacilli and other aciduric micro-organisms (c. 10^1–10^2 ml^{-1} of content), generally from the mouth. However, in some individuals, *H. pylori* colonizes the gastric mucosa (Gottlieb *et al.* 1993; Williams 2001), and carriers may be symptomatic or nonsymptomatic. A short residence time of about 1–2 h, gastric acid and nitric oxide, which arises from bacterial reduction of salivary nitrate, are the main factors preventing significant microbial growth in the stomach. Studies have shown that a pH of lower than 4 is effective in limiting the growth of bacteria (Gianella *et al.* 1972). The upper small bowel principally contains lactobacilli and streptococci (Lin *et al.* 1995) in numbers slightly higher than those found in the stomach (10^2–10^4 ml^{-1} of aspirate).

Patients with dysphagia, such as individuals suffering from oropharyngeal or neurological illnesses may need enteral feeding. This has been shown to maintain gut function more effectively than parenteral nutrition, which can lead to severe gut dysfunction. Percutaneous endoscopic gastrostomy (PEG) feeding is a common form of enteral nutrition widely employed in long-term support, in which the feeding tube passes through the abdomen into the stomach. However, diarrhoea is a common complication in patients with PEG tubes associated with enteral nutrition, together with aspiration pneumonia and stoma infections (Cabre and Gassull 1993).

Extensive microbial biofilms form on the surfaces of PEG, when they are placed in the body (O'May *et al.* 2005a,b). Table 1 shows a comparison between the major culturable micro-organisms in gastric aspirates and in PEG tube biofilms (O'May *et al.* 2005b). It can be seen that there is significant microbial overgrowth in the stomachs of PEG tube patients, and that the overwhelming majority of these organisms are facultative anaerobes. Moreover, many of these species are potential pathogens. Planktonic species such as bifidobacteria and klebsiella were not found in the biofilms, while the reverse was the case with enterococci, campylobacters, corynebacteria and the yeast kloeckera. Interestingly, some of the patients involved in the study had been receiving antibiotics, and staphylococci, *Escherichia coli* and candidas were only isolated from these individuals.

Table 1 Incidence and cell population sizes of the predominant microbial communities during microbial overgrowth in PEG tube patients*

	Log_{10} viable cells ml^{-1}	
Organisms ($n = 10$)	Aspirates ($n = 22$)	PEG tube biofilms
Bifidobacteria	4·9 (3)†	ND
Lactobacilli	4·9 (8)	6·5 (6)
Staphylococci	5·1 (8)	4·3 (4)
Streptococci	5·1 (7)	4·8 (3)
Enterococci	ND	6·0 (6)
Escherichia	4·8 (9)	4·8 (6)
Klebsiella	5·3 (3)	ND
Campylobacters	ND	6·3 (2)
Corynebacteria	ND	4·8 (5)
Candidas	4·2 (6)	4·7 (6)
Kloeckera	ND	4·9 (2)

ND, Not detected.
*Results adapted from O'May *et al.* (2005b).
†Values in parentheses show the number of patients from whom the organisms were isolated.

Growth of bacteria in the small bowel

Under normal circumstances, transit time of digesta through the human small intestine is between 2 and 4 h, and it this rapid flow of material that prevents permanent microbial colonization of the upper gut (Macfarlane and Cummings 1991). However, bacterial numbers increase progressively as intestinal contents pass along the bowel, and at the ileo-caecal valve, significant populations of what are recognizably faecal bacteria become established, with cell counts in the region of 10^8–10^9 g^{-1} of gut contents (Macfarlane *et al.* 1995). Under certain circumstances, significant microbial overgrowth can occur in the small bowel and stomach, caused by a number of factors such as achlorhydria, reduced gut motility, strictures, radiation treatment, drugs, antibiotics and small bowel resection. In contaminated small bowel syndrome, high numbers of bacteria become established in the upper digestive tract, often with severe physiological consequences for the host, such as diarrhoea which can lead to electrolyte loss, iron deficiency caused by mucosal bleeding, reduced brush border enzyme activities, vitamin B_{12} deficiency resulting from microbial uptake, steatorrhoea caused by bacterial bile acid deconjugation, and a general malabsorption of nutrients. As shown in Fig. 4, microbial overgrowth in the upper gut can also result in increased gut permeability, resulting in septic morbidity and organ failure.

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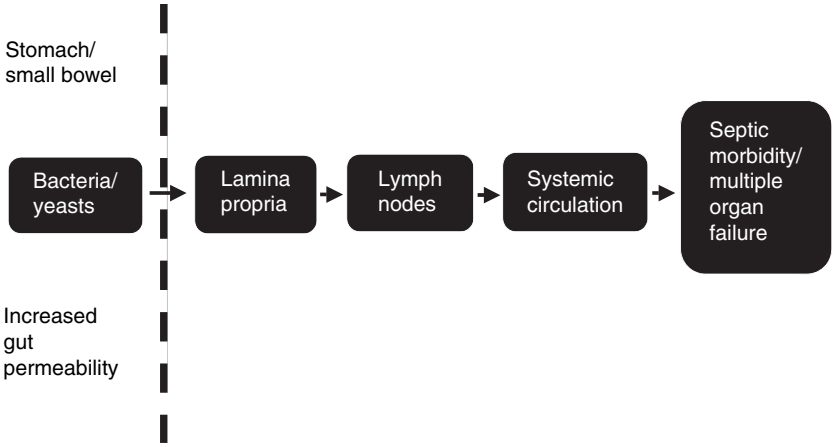

Figure 4 Consequences of microbial overgrowth in the upper gastrointestinal tract.

The large intestine

Lumenal biofilm communities

Largely because of the anatomy of the large intestine, the physiology of host digestive processes and the mechanics of movement of particulate substances through the gut, bacteria that are able to rapidly colonize food residues in the caecum serve as inocula for new food residues entering the colon. These organisms are likely to be of particular ecological importance in maintaining stability in the large intestinal microbiota. Few investigations have looked at the way colonization of particulate substances takes place in the gut, but it is likely that the bacteria involved in the initial stages of this process form biofilms.

Recent work has shown that bacterial populations strongly adhering to particulate matter in stools were phenotypically similar in composition to unattached communities, with bacteroides and bifidobacteria predominating (Macfarlane and Macfarlane 2006). The biofilms comprised a mixture of living and dead bacteria, and confocal laser scanning microscopy showed that bacteria occurred as isolated dispersed cells and in microcolonies at the interface with the substratum. Fermentation experiments with a variety of complex carbohydrates demonstrated that biofilm populations were more efficient in digesting polysaccharides, while nonadhering communities broke down oligosaccharides most rapidly. Acetate was the principal fermentation product formed by biofilm bacteria, whereas higher levels of butyrate were produced by nonadherent populations, showing that the two communities could be distinguished in metabolic terms.

Enzyme measurements had previously indicated that biofilms occurring on digestive substances in the gut lumen formed metabolically distinct assemblages in the ecosystem, with respect to the breakdown and metabolism of mucins and other complex macromolecules (Macfarlane *et al.* 1997). It was shown in these studies that with the exception of *N*-acetyl α-galactosaminidase, the vast majority of mucinolytic glycosidases were cell-associated in faecal material. Although little difference in β-galactosidase and *N*-acetyl β-glucosaminidase activities was found in the biofilms, the expression of α-fucosidase and *N*-acetyl α-galactosaminidase activities was considerably lower than in nonadherent bacteria. Proteases and peptidases are also involved in mucin degradation, and measurements of these hydrolytic enzymes in biofilm and nonadherent communities, using a range of protease inhibitors, indicated that while the spectrum of proteolytic/peptidolytic activity was essentially the same in relation to the formation of serine, thiol and aspartic proteases, there were variations in chymotrypsin and trypsin, and to a lesser degree, metalloprotease activities. The higher trypsin and chymotrypsin in the biofilms were attributed to adsorbed pancreatic endopeptidases; however, lower metalloprotease activities could only be due to differences in bacterial enzyme expression.

Mucosal biofilm populations

Unlike the situation in humans, mucosal bacterial communities in the GI tracts of animals have been well characterized, and specific microbiotas have been identified on gut epithelial surfaces (Wallace *et al.* 1979; Lee 1980). However, some early human studies suggested that mucosal populations are generally similar to those present in the gut lumen (Nelson and Mata 1970). Bacteroides were reported to occur on the mucosal surface, but other gut anaerobes including eubacteria, bifidobacteria, clostridia and a variety of Gram-positive cocci were also detected (Edmiston *et al.* 1982; Croucher *et al.* 1983).

The structure and composition of bacterial communities inhabiting the epithelial surface, as well as those existing in the mucus layer, are likely to be determined by a variety of host factors, including humoral immunity and elements of the innate immune system such as defensins,

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which are antimicrobial peptides formed by epithelial cells, that are active against viruses, fungi, Gram-positive and Gram-negative bacteria (Mahida *et al.* 1997). The rate of synthesis and chemical composition of mucus, epithelial turnover rates, diet, availability of adhesion sites, lysozyme production, pancreatic endopeptidases, colonization resistance mediated by the normal commensal microbiota and gut motility are also important factors affecting biofilm community structure.

Molecular analysis of mucosal populations on colonic tissue by Hold *et al.* (2002) showed that 85–89% of the bacteria occurred in the three same phylogenetic groups that were found when faecal material was cloned (Suau *et al.* 1999). No bifidobacteria were detected in the latter study, which has also been the case in other cloning experiments with faeces (Wilson and Blitchington 1996; Suau *et al.* 1999), indicating the importance of primer selection in these studies. Twenty eight per cent of the sequences recovered were less than 97% related to known bacteria in databases, indicating that a significant part of the microbiota is unknown. Zoetendal *et al.* (2002) used PCR with denaturing gradient gel electrophoresis to study tissues taken from the ascending, transverse and descending colons of ten people, in comparison to faecal material. It was reported that the microbiotas were host-specific, and that the organisms were uniformly distributed along the colon. Moreover, mucosal biofilms differed significantly in composition to bacterial communities in faecal material.

Microscopic investigation of the three dimensional structure of colonic biofilms has shown that mucosal bacteria are distributed throughout the mucus layer, but that they are not usually found in healthy crypts (Macfarlane *et al.* 2004). Using specific 16S rRNA fluorescence *in situ* hybridization probes, this study further demonstrated that many bacteria, including enterococci, bacteroides and bifidobacteria occurred extensively in microcolonies. Live/dead staining of these structures showed that most of the bacteria were living, particularly cells that were close to the mucosal surface. These findings suggested that the bacteria were actively growing in the mucus layer, and that their presence was not a result of passive transference of the cells from faecal material in the gut lumen. The presence of immunogenic bacterial species in microcolonies on epithelial surfaces may have implications for some forms of gut disease, such as ulcerative colitis (UC), as greater numbers of bacteria would give rise to higher localized concentrations of bacterial antigens, toxins or other harmful secretory products.

Ulcerative colitis

Many of the diseases that occur in the human large intestine are of unknown aetiology, but micro-organisms have been implicated either as causative agents or maintenance factors in many bowel disorders. A number of bacterial species perturb normal gut homeostasis and evoke an acute inflammatory response in the host. In most, although not all cases, the principal organisms involved are adherent or invasive to the gut epithelium and include enterotoxigenic strains of *E. coli*, as well as species belonging to the genera *Yersinia, Shigella, Salmonella, Campylobacter* and *Aeromonas* (Cohen and Giannella 1991). The clinical effects of these bacteria are usually acute rather than chronic, and their pathogenicity and host responses to infection have been well studied.

The inflammatory response of UC is primarily located in the colonic mucosa and submucosa. The distal colon is always affected, with the condition expressing itself in acute attacks followed by periods of symptom-free remission. The disease almost invariably appears first in the rectum and distal colon, and over time, it usually progresses up the colon towards the proximal bowel. Bacterial involvement has been proposed in both the initiation and maintenance stages of UC (Cummings *et al.* 2003). Sulfate-reducing bacteria (SRB) belonging to the genus *Desulfovibrio* have been studied extensively in relation to their involvement in the initiation and/or maintenance of UC (Gibson *et al.* 1991; Zinkevich and Beech 2000; Loubinoux *et al.* 2002), principally through their production of sulphide, which is highly toxic to colonic epithelial cells. However, recent molecular studies have shown that there is little difference in mucosal SRB carriage rates in healthy people and UC patients (Fite *et al.* 2004), suggesting that if sulfide is involved in UC, host defects in its detoxication pathways are probably responsible. A number of bacterial pathogens including bacteroides, fusobacteria, *Streptococcus mobilis* and shigellas have all been considered to be aetiologic agents in this form of colitis (Onderdonk 1983), partly because some of these organisms are able to penetrate the gut epithelium, or induce similar disease symptoms in experimental animals. It has also been reported that some strains of *E. coli* isolated from the colitic bowel have increased adhesive properties (Chadwick 1991), although this may be an adaptation to inflammatory changes and tissue destruction at the mucosal surface. Macfarlane *et al.* (2004) observed only slightly higher than normal levels of *E. coli* and other facultative anaerobes in mucosal tissue taken from UC patients; however, Onderdonk and Bartlett (1979) reported that antimicrobial agents that were specifically active against obligate anaerobes prevented mucosal ulceration in the guinea pig model of colitis, while Monteiro *et al.* (1971) found increased antibody production against strictly anaerobic species. In general, however, evidence for a specific transmissible agent in UC is weak, as antibody production is usually low and the majority of

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organisms linked to the disease by various workers are not found in all patients. To date, therefore, Koch's postulates have not been fulfilled for UC.

Nevertheless, there is still support for the idea of mucosal bacteria being linked to the aetiology of UC, either by pathogenic organisms colonizing the epithelial surface and invading the underlying mucosa, or alternatively, by species belonging to the normal healthy mucosal community occupying adhesion sites on the gut surface and preventing attachment and growth of harmful organisms. Moreover, as bacteria growing on the gut mucosa exist in close juxtaposition to host tissues, intuitively, it might be expected that these organisms interact to a greater extent with the host immune and neuroendocrine systems than their lumenal counterparts.

Mucosal bacterial populations in UC

One way to avoid the need to cleanse mucosal tissues before biopsy is to use rectal biopsies, which are more readily available from gastroenterology out-patients clinics, while rectal inflammatory manifestations are easily recognizable by the physician. Rectal biopsies were taken during endoscopy from nine colitic patients, and ten control subjects who had no detectable inflammatory bowel disease, in a study to compare mucosal biofilm communities (Macfarlane *et al.* 2004). Complex bacterial communities were found to colonize the mucosal surface in all of the volunteers, as summarized in Table 2. In total, 72 different types of bacteria belonging to 18 bacterial genera were identified in this investigation, with only 20 species being found to be common to both groups. However, statistical analysis showed that only differences in bifidobacterial populations were significant. Several different bifidobacterial species were identified on the mucosa, and marked quantitative and qualitative variations in these organisms were observed in both subject groups. Bifidobacteria were present in considerably lower numbers in UC, and the apparent absence of *Bifidobacterium adolescentis* in this group was notable. Other differences were apparent, including the fact that only *Bifidobacterium angulatum* and *Bifidobacterium bifidum* were isolated from both groups of patients. Low numbers of bifidobacteria, or the absence of particular bifidobacterial species on the mucosa may be of significance in inflammatory bowel disease. Some bifidobacterial species have well-documented immunomodulatory properties (Macfarlane and McBain 1999), and together with lactobacilli, these bacteria are thought to contribute towards host defences in the gut (Famularo *et al.* 1997), through interactions with the immune system (De Simone *et al.* 1992; Furrie *et al.* 2005), and colonization resistance (Faure *et al.* 1984; Araya-Kojima *et al.* 1995; Gill *et al.* 2001). However, in the investigation by Macfarlane *et al.* (2004), lactobacillus numbers were low in both controls and UC patients, and no significant differences were found in UC, although reduced numbers of these organisms have been observed in pouchitis patients (Ruseler van Embden *et al.* 1994). Peptostreptococci were only detected in people who had UC, who also had proportionally more facultative anaerobes than found in the control subjects. Microscopy showed that bacteria on the rectal mucosa often occurred in microcolonies, which seems to be a characteristic of mucosal communities throughout the GI tract. Interindividual variations in mucosal biofilms made it difficult to assign a role for specific bacteria in UC aetiology; however, the strong differences seen in bifidobacteria and peptostreptococci may implicate these organisms in this disease.

Manipulation of colonic mucosal communities through diet

Research has shown that the composition of mucosal biofilms can change markedly in inflammatory conditions such as UC and Crohn's disease (Macfarlane *et al.* 2004; Prindiville *et al.* 2004). Very little is known of the significance of diet on these gut biofilms; however, a few studies

Table 2 Major groups of bacteria isolated from rectal biopsies in healthy subjects and UC patients*

	Controls		UC	
Bacteria†	Range	Mean	Range	Mean
Total bacteroides	2·0–7·0 (10)	5·2 ± 1·4	4·4–6·2 (6)	5·2 ± 0·7
Total bifidobacteria	3·5–6·0 (8)	5·1 ± 1·0	2·6–4·7 (8)	3·6 ± 0·7
Total prevotellas	4·5–5·9 (4)	5·2 ± 0·7	2·6–3·1 (2)	2·9 ± 0·4
Total Gram-positive cocci	3·7–5·5 (7)	4·4 ± 0·7	1·8–5·4 (8)	4·2 ± 1·4
Total Gram-negative cocci	1·8–5·2 (4)	3·3 ± 1·7	4·3–5·2 (3)	4·6 ± 0·5
Enterobacteria	1·5–6·3 (8)	3·5 ± 1·8	2·2–4·7 (8)	4·2 ± 0·8

*Data are from Macfarlane *et al.* (2004).

†Results are log cell counts cm^2 of rectal tissue ± standard deviation. Values in parentheses show the number of subjects positive for carriage of the bacteria.

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Table 3 Effects of dietary supplementation with inulin and oligofructose on mucosal biofilm communities in the distal large bowel in a human feeding trial*

Bacteria†	Control	With prebiotic
Total anaerobes	8·7 ± 0·1	8·6 ± 0·1
Facultative anaerobes	6·4 ± 0·3	5·9 ± 0·4
Bifidobacteria	5·2 ± 0·3	6·4 ± 0·3‡
Eubacteria	4·6 ± 0·3	6·1 ± 0·3‡
Clostridia	5·0 ± 0·3	4·9 ± 0·3
Lactobacilli	3·1 ± 0·1	3·6 ± 0·2
Bacteroides	8·3 ± 0·2	8·5 ± 0·2
Enterobacteria	6·4 ± 0·3	5·9 ± 0·4

*Results are from Langlands *et al.* (2004). Subjects ($n = 14$) were fed a mixture comprising 7·5 g oligofructose and 7·5 g inulin per day for 2 weeks, or were not given anything ($n = 15$).

†Values are $\log_{10}$ bacterial numbers (gram of mucosal tissue)$^{-1}$ ± SEM.

‡Prebiotic effects that were significantly different from their respective controls ($P < 0·05$).

have shown that the microbial composition of mucosal communities in humans can be manipulated through the use of prebiotics. Langlands *et al.* (2004) showed that bifidobacterial and eubacterial numbers could be increased more than tenfold in mucosae of the proximal and distal colons in patients fed 15 g of a prebiotic mixture containing 7·5 g inulin and 7·5 g FOS per day for 2 weeks prior to colonoscopy (Table 3). Potential mechanisms whereby dietary components in the gut lumen can affect bacteria on the mucosal surface include cross-feeding of small oligosaccharides formed during the hydrolysis of complex polysaccharides by lumenal bacteria, production of electron sink compounds such as lactate, succinate and hydrogen by the lumenal microbiota, which are used as electron donors by bacteria growing on the epithelial surface. However, until this study, it was unclear whether bacteria growing in mucosal biofilms could sequester dietary components, or whether they were principally dependent on mucus and other host secretions. Importantly, the investigation demonstrated that even small dietary changes could have profound effects on the mucosal microbiota, which opens up the possibility that new therapeutic strategies can be developed for tackling bacteria-associated gut diseases.

Very few clinical trials have been undertaken on the use of probiotics in inflammatory bowel disease, nevertheless, as is often the case with any potentially new treatment, there is considerable optimism for the introduction of probiotic therapies. In two human investigations, a nonpathogenic strain of *E. coli* (Nissle 1917) has been compared with the anti-inflammatory drug mesalazine in UC patients who were either in remission (Kruis *et al.* 1997), over 12 weeks, or after relapse (Rembacken *et al.* 1999) over 12 months. In both investigations, it was reported that the probiotic was as useful as the drug in preventing relapse. In another uncontrolled trial involving 20 mesalazine intolerant UC patients in remission, the subjects were given a mixture of eight probiotic bacteria. The results showed that 15 of the 20 patients were still in remission after 1 year (Venturi *et al.* 1999).

Furrie *et al.* (2005) reported a double-blinded randomized controlled trial in which a synbiotic was fed to UC patients, with active disease, for a period of 1 month. Eighteen individuals were enrolled into the investigation, and those receiving the synbiotic were given 12 g of Synergy 1, which is an oligofructose-enriched inulin, together with 2×10^{11} live *Bifidobacterium longum* per day. This strain had previously been isolated from a healthy rectal mucosa, and selected on the basis of its anti-inflammatory properties, using the HT-29 human cell line. The results showed that bifidobacterial numbers on the rectal mucosa increased 42-fold in subjects receiving the synbiotic, and this was accompanied by highly significant reductions in mucosal pro-inflammatory cytokines (TNF-α, IL-1α) as well as inducible β-defensins 2, 3 and 4, which are markers of inflammation in epithelial cells. Histology further showed resolution of inflammation and abscess formation in patients who had taken the synbiotic.

Conclusions

Because relatively few investigations have been carried out on microbial biofilms in the human digestive tract, little is really known about the structure and function of these entities, and with the possible exception of their interactions with the innate immune system, their metabolic and neuropathological significance to the host. To a large extent, studies on bacterial growth in biofilms in the human body are still in their infancy, and until recently, the analytical tools that have been used to study biofilm composition and metabolism have been innately destructive, and have not provided much information on spatial organization, or the way different groups of organisms interact with each other. The acquisition of fresh tissue samples will always be problematic; however, emerging technologies will improve our understanding of the temporal, metabolic and spatial organization of these microcosms. The increasing shift in emphasis away from culture-based studies, and further development of molecular techniques (Hold *et al.* 2002; Aminov *et al.* 2006), together with the emergence of methodologies for investigating gene expression *in situ* (Hoshino *et al.* 2001), will greatly facilitate future work on biofilm structures in the GI tract.

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