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A tale of two habitats: *Bacteroides fragilis*, a lethal pathogen and resident in the human gastrointestinal microbiome

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Abstract

Bacteroides fragilis is an obligately anaerobic Gram-negative bacterium and a major colonizer of the human large colon where *Bacteroides* is a predominant genus. During the growth of an individual clonal population, an astonishing number of reversible DNA inversion events occur, driving within-strain diversity. Additionally, the *B. fragilis* pan-genome contains a large pool of diverse polysaccharide biosynthesis loci, DNA restriction/modification systems and polysaccharide utilization loci, which generates remarkable between-strain diversity. Diversity clearly contributes to the success of *B. fragilis* within its normal habitat of the gastrointestinal (GI) tract and during infection in the extra-intestinal host environment. Within the GI tract, *B. fragilis* is usually symbiotic, for example providing localized nutrients for the gut epithelium, but *B. fragilis* within the GI tract may not always be benign. Metalloprotease toxin production is strongly associated with colorectal cancer. *B. fragilis* is unique amongst bacteria; some strains export a protein >99% structurally similar to human ubiquitin and antigenically cross-reactive, which suggests a link to autoimmune diseases. *B. fragilis* is not a primary invasive enteric pathogen; however, if colonic contents contaminate the extra-intestinal host environment, it successfully adapts to this new habitat and causes infection; classically peritoneal infection arising from rupture of an inflamed appendix or GI surgery, which if untreated, can progress to bacteraemia and death. In this review selected aspects of *B. fragilis* adaptation to the different habitats of the GI tract and the extra-intestinal host environment are considered, along with the considerable challenges faced when studying this highly variable bacterium.

INTRODUCTION: THE MICROBIOTA AND BEYOND

The introduction of routine anaerobic culture in clinical diagnosis and eventual recognition of the need for treatment of anaerobic infection in the 1970s identified *Bacteroides fragilis* as the most common Gram-negative anaerobic bacterium isolated from infection (reviewed in [1, 2]). The first description of the involvement of *B. fragilis* (originally *Bacillus fragilis*) in infection was, however, in the late 1870s [3]. The discovery that metronidazole provides effective treatment and surgical prophylaxis [4] greatly improved clinical outcomes and reduced patient mortality. Until recently, resistance to metronidazole has remained low and this may explain reduced interest in the virulence mechanisms of *B. fragilis* and a lack of recognition of its importance as a pathogen. *B. fragilis* is still, however, a potentially lethal pathogen with mortality from bacteraemia of up to 19% despite treatment [5]. A further worrying development is multidrug-resistant *B. fragilis* infection, which, for example, resulted in the death of a patient after routine gall bladder surgery [6]. *B. fragilis* is not, however, a classical faecal–oral transmitted primary enteric pathogen; it is a normal gut resident. (The definitions of 'microbiota' and 'microbiome' as used within this review are detailed in Box 1.) Interest in the role of *Bacteroides* in the human resident gastrointestinal (GI) tract microbiota has increased with availability of rapid and relatively inexpensive high-capacity DNA sequencing; it is a predominant genus in the large intestine, metagenomic sequencing [7] having confirmed earlier culture data (e.g. [8, 9]). It should be noted that *Prevotella* spp., also members of the phylum Bacteroidota (Bacteroidetes) [10, 11], and *Bacteroides* spp. in general, are the most abundant GI tract genera and that factors, including diet, can influence the relative abundance of either genus. Study of *Bacteroides* in the GI tract has revealed multiple complex host interactions, which can be benign, beneficial or lead to the development of GI tract pathologies such as colorectal cancer and inflammatory conditions [12]. Detailed discussion of the role of *B. fragilis* in GI tract pathologies is outwith

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Abbreviations: BfUbb, *Bacteroides fragilis* ubiquitin; GI, gastrointestinal; PUL, polysaccharide utilisation locus.
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Box 1. Definitions and usage

Microbiome: 'a characteristic microbial community occupying a reasonable well-defined habitat which has distinct physico-chemical properties'[1] inclusive of archaea, bacteria, microbial eukaryotes and viruses; their genomes and their habitat[2].

Microbiota: the living organisms present within a defined environment, generally considered to exclude viruses, bacteriophages, prions and nucleic acids not within microbial cells[1].

Microflora: (misnomer) should not be used, microbes are not plants.

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2. Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal. *Microbiome*. 2015;3:31. doi:10.1186/s40168-015-0094-5 [75].

the scope of this review; however, recent studies, including topics which were presented at the Microbiology Society Anaerobe 2021 Focused Meeting, are put into the context of earlier work. In addition, challenges relating to the study of *B. fragilis*, an unusual and fascinating bacterium, are highlighted.

B. fragilis in the gastrointestinal microbiome

Colonization of the human GI tract by *Bacteroides* spp., including *B. fragilis*, occurs in the early neonatal period, from the mother and other individuals who interact closely with the baby [13, 14] . With time, the microbial community becomes relatively stable, being shaped by diet and lifestyle [15]. Under normal circumstances colonization is benign and potentially beneficial. Defining the bacterial content of GI tract microbiota by DNA sequencing can be problematic due to technical issues of the selective loss of DNA from some types of bacteria, including *Bacteroides* spp., during sample storage [16]. There is strong evidence, however, that *Bacteroides* is a predominant genus in the GI tract [7], with conservative estimates of 10^9 and up to 10^{11} cells of the genus *Bacteroides* g^{-1} faeces. It should be noted that some of the higher early literature estimates of the numbers of bacteria in faeces may relate to dry weight rather than wet weight. *B. fragilis* detection is dependent on the nature of the sample (faecal or mucosal), sample size and culture conditions, although the consensus from culture suggests that most people may be colonized. Analysis of metagenomic data may help to resolve the carriage rate of *B. fragilis* in humans, but the same caveats relating to samples apply, as well as sample storage conditions, as discussed above. Faecal analyses by culture indicate that *B. fragilis* is not the predominant *Bacteroides* species. Estimates vary, but *B. fragilis* may account for between 1–14% of the *Bacteroides* species in faeces, whereas other *Bacteroides* and related species of the Bacteroidota (Bacteroidetes) are considered to be more numerous [9]. These are percentage values of 10^9 or more bacteria g^{-1} faeces, therefore despite not being the predominant *Bacteroides* species, *B. fragilis* can be a major component of the faecal microbiota, with potentially billions of *B. fragilis* within the GI tract.

Digestive help

Breakdown of plant polysaccharides that the human digestive tract is incapable of degrading and the production of short-chain fatty acids, which are used as an energy source by the intestinal epithelium, are key activities of *Bacteroides* spp. within the GI tract habitat [17]. In addition, *Bacteroides* species, including *B. fragilis*, degrade host-derived mucosal glycans, including mucins and the *N*- linked oligosaccharides of glycoproteins [18]. Mucin in the large colon forms a protective layer over the GI tract epithelium, which is replenished by secretion from goblet cells. The inner mucin layer adjacent to the epithelium is normally sterile [19]. A subset of the microbiota, which includes *B. fragilis*, are capable of degrading mucin and graze at the gut lumen edge, with initial degradation involving sialidase and endo-mucinase activity [20]. *Bacteroides* species biofilms directly adjacent to the mucosal surface may be an indication of abnormality and have been observed in patients with inflammatory bowel disease [21].

The gene clusters relating to glycan degradation, known as polysaccharide utilization loci (PULs), characteristically contain a pair of genes encoding a Ton-B dependent porin, *susC* (**s**tarch **u**tilization **s**ystem) and a glycan-binding protein, *susD*. These genes are related to the *ragA*/*ragB* genes described in *Porphyromonas gingivalis*, which are associated with periodontal destruction [22]. Multiple putative *susC* homologues are present in the *B. fragilis* genomes; in the *B. fragilis* type strains NCTC 9343 (aka ATCC 25285) there are two complex intergenic shufflons that contain five and four *susC/D* gene pairs, respectively. Complex DNA inversions from recombination between repeat regions bring the alternative *susC/D* genes downstream of a promoter. Evidence for these DNA inversion events was evident in the shotgun DNA sequences in the first *B. fragilis* complete genome sequencing project [23]. A further level of variation was also evident for one of the shufflons in which the promoter was within an independent invertible DNA region. Inversion of the DNA region containing the promoter reversibly switches transcription of the downstream genes off and on. Invertible promoters were also identified upstream of several putative *susC* homologues not located within intergenic shufflons, as well as a gene cluster related to sialoglycoconjugate degradation. Invertible promoter regions, under the control of multiple different site-specific recombinases, control the transcription of a wide range of genes and loci in *B. fragilis*, including DNA restriction/modification and multiple polysaccharide biosynthesis operons (Fig. 1) [23].

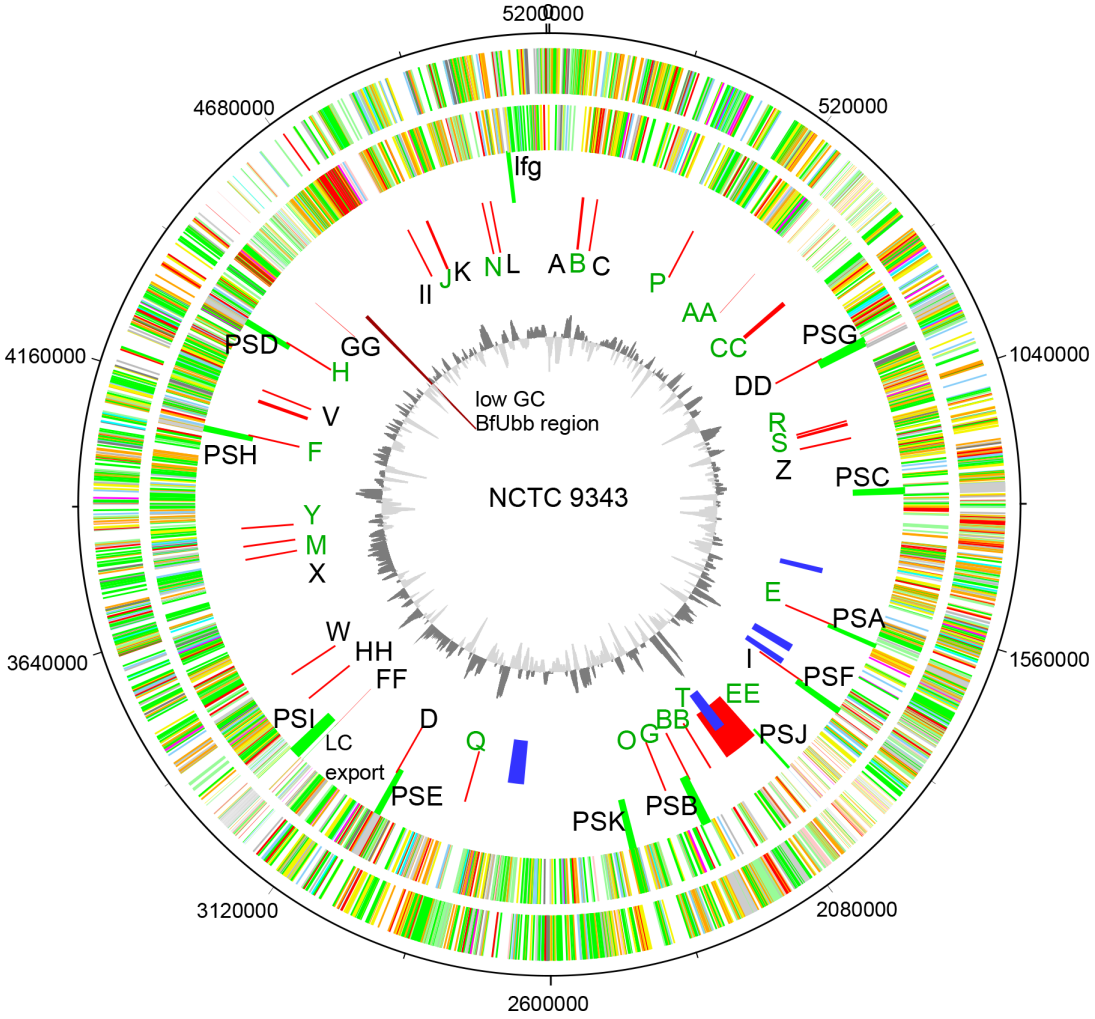

Fig. 1. Circular representation of the *B. fragilis* NCTC9343 chromosome to illustrate the position of invertible DNA regions and polysaccharide synthesis-associated regions. From the outside: circle 1, DNA coordinates; circles 2 and 3, CD forward/reverse strands; circle 4, surface polysaccharide (PS) synthesis-associated loci, large capsule export-associated genes (LC export), *lfg*, protein glycosylation locus; circle 5, invertible DNA regions, green text indicates active inversion sites where DNA was present in more than one orientation within the first complete NCTC9343 shotgun sequence [23], BB, CC and EE designate shufflons with multiple complex DNA inversions; circle 6, putative integrative and conjugative elements, note the putative conjugative transposon within the active shufflon EE; circle 7, graph of %GC content, dark grey above genome average, light grey below average, low-GC BfUbb region indicates the region containing the gene for *B. fragilis* ubiquitin. Colour coding for circles 2 and 3: white, pathogenicity/adaptation; dark grey, energy metabolism; red, information transfer (transcription/translation, DNA modification); dark green, surface-associated; dark blue, RNA; sky blue; degradation of large molecules; dark pink; degradation of small molecules; yellow, central/intermediary metabolism; light green, unknown; light blue, regulators; orange; conserved hypothetical, pink; phage/ insertion sequence elements; light grey, miscellaneous. Image generated using Artemis DNA plotter [76].

Autoimmune disease and a ubiquitin molecular mimic

The resident microbiota is inextricably linked to the human immune system; it is considered to be necessary for normal immune system development but may also have potentially adverse impacts on health (reviewed in [1, 24]). There is increasing recognition of the potential involvement of the GI tract microbiota in autoimmune disease [25]. Development of autoimmune disease is considered to be a combination of genetic background and environmental influences. A range of generic environmental factors, such as lifestyle (e.g. diet, exercise, stress) and exposure to as yet unidentified infectious agents or pollutants, have been considered [26], but to date none have been linked definitively with the development of autoimmune disease. Any of these generic environmental factors could impact on the GI tract microbiome or breach the integrity of the gut epithelium, exposing the immune system to microbiome components. It should also be taken into consideration that the microbiota is inherited from not just family but also other individuals in close contact with the infant [13, 14, 27]. The increased incidence of autoimmune and inflammatory bowel conditions observed in some next-generation immigrants, thought to be caused by 'environmental factors'

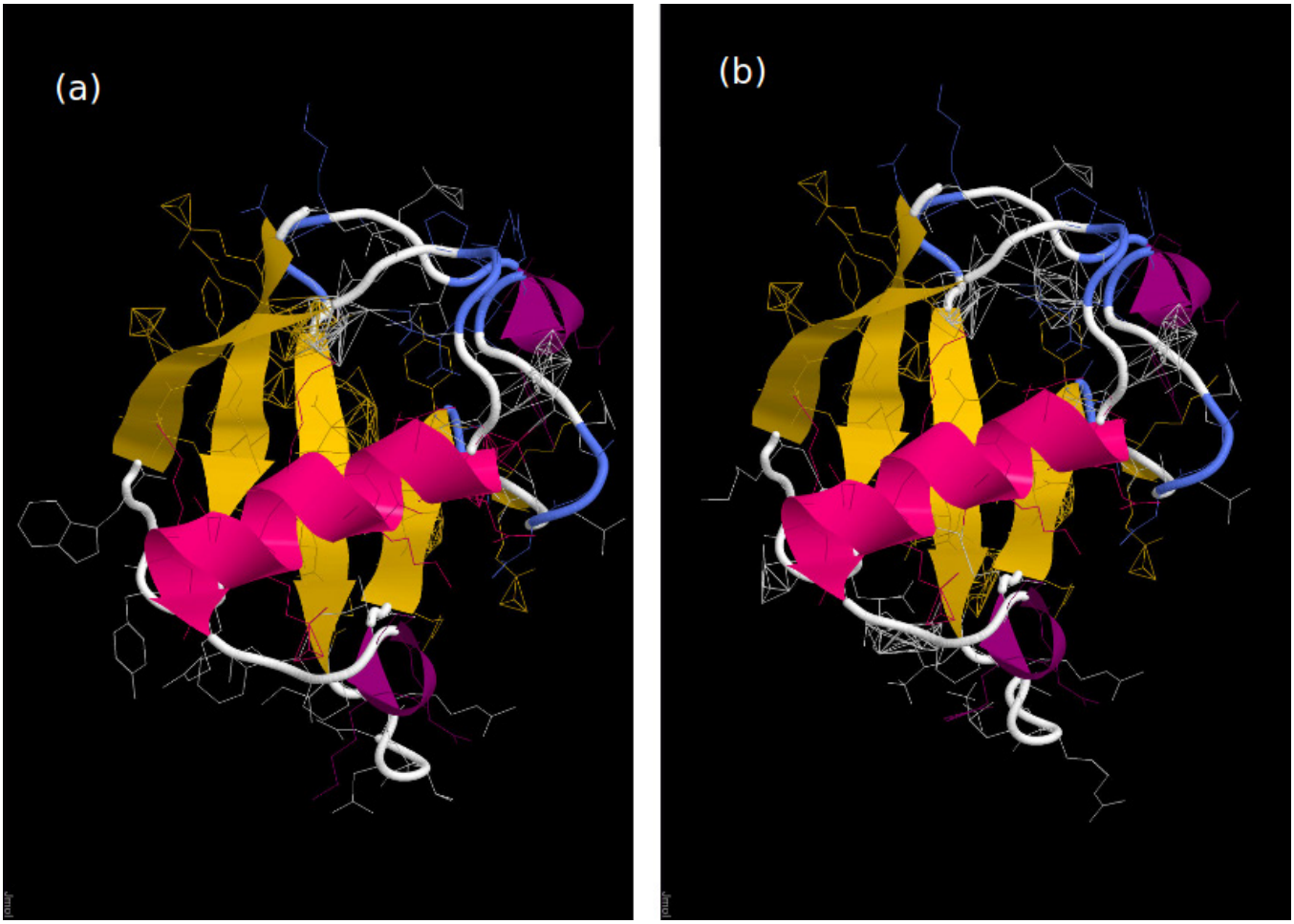

Fig. 2. Structural comparison of (a) *Bacteroides fragilis* ubiquitin, BfUbb, and (b) human ubiquitin generated with the Protein Homology/analogY Recognition Engine [77].

[28], could relate to colonization of infants with microbiota strains from the local population. Familial disease associations may be a combination of the inheritance of genetic predisposition and microbiota inheritance from family and other individuals in close contact with the infant.

Molecular mimicry is hypothesized to be a trigger for the development of autoimmunity [29]. Exposure of the immune system to exogenous molecules with a sufficiently similar structure may either break tolerance to self-antigens or activate pre-existing but unreactive immune system cells. *B. fragilis* is unique amongst bacteria; a gene that encodes a homologue of human ubiquitin was discovered in the *B. fragilis* NCTC 9343 genome [30]. *B. fragilis* ubiquitin protein (BfUbb) is 63% identical and 84% similar to the human ubiquitin amino acid sequence; it is >99% structurally similar to human ubiquitin by protein modelling prediction and analyses (Fig. 2) [30, 31]. It is likely that the gene entered the *B. fragilis* genome via horizontal gene transfer; it is located within an 11 kb region of DNA with a lower %GC content than the average for the *B. fragilis* genome (Fig. 1) [32]. In all eukaryotes tagging of proteins and other molecules by ubiquitin is an absolute necessity for normal functioning of the organism. Functions dependent on ubiquitin include regulation of proteolysis; membrane protein endocytosis and trafficking; transcription; DNA repair; antigen presentation and immune system development and function [33–35]. The amino acid sequence of eukaryotic ubiquitin is highly conserved and identical in mammals. Prokaryotes have ubiquitin-like systems but lack the conserved ubiquitin protein of eukaryotes [36]. The BfUbb gene has evolved a key new feature; an *N*-terminal signal sequence that directs BfUbb export from the bacterial cytoplasm [30]. BfUbb therefore does not appear to have a function inside the bacterial cell and is detectable in bacterial culture supernatants associated with outer-membrane vesicles. The primary role of BfUbb within the microbiome is likely antimicrobial activity [37]. The structural similarity of BfUbb and human ubiquitin is consistent with immunological cross-reactivity of *B. fragilis* and mammalian ubiquitin [31].

Investigation of serum from patients referred for first-time testing to an immunology diagnostic laboratory for autoimmune disease revealed that significantly more individuals with a diagnostic test indicative of connective tissue disease (e.g. lupus), rheumatoid arthritis and coeliac disease had higher levels of IgG antibodies to BfUbb than healthy volunteers. Some individuals had IgG that reacted with *both* BfUbb and human ubiquitin. Antibody cross-reactivity profiling shows that BfUbb has both unique regions and human ubiquitin cross-reactive epitopes [31]. It could be that molecular mimicry by BfUbb breaks self-tolerance to ubiquitin and potentially other self-proteins to which ubiquitin is bound, leading to the generation of antibodies against self-epitopes. The consequences of circulating serum antibodies reactive with BfUbb are unknown; however, the levels of antibodies cross-reactive with human ubiquitin could clearly be significant for health.

In addition to the potential for the activation of an autoimmune response, BfUbb can interfere directly with ubiquitin pathways. Purified BfUbb can bind to the human E1-activating enzyme and inhibits ubiquitination in laboratory experiments [30]. Misregulation of ubiquitin is implicated in immune system disfunction and other diseases, including cancer, inflammatory bowel disease, neurogenerative disorders and type 2 diabetes. Future research may reveal that BfUbb plays a role in conditions for which the root cause is currently unknown by direct interference with normal ubiquitin functions as well as potentially triggering autoimmune disease.

B. fragilis infection: from abscesses to bacteraemia

A stable GI tract community provides a high level of protection from primary intestinal pathogens such as *Salmonella* spp., which, to cause disease, have to outcompete the residents for nutrients and establish in sufficient numbers to generate pathological change [38]. It may seem counter-intuitive that a member of the GI tract community has the potential to become a lethal pathogen if given the opportunity to enter into extra-intestinal habitats within the host. Indeed, only a select few of the diverse members of the normal resident GI tract microbiota become potentially lethal pathogens in healthy individuals. Infections arise when *B. fragilis* escapes from its normal habitat of the GI tract to other body sites as a result of damage to intestinal integrity. Such damage can arise from rupture of an inflamed appendix, GI tract surgery, physical injury (e.g. gunshot wounds), colonic cancer and diverticulitis [39]. Gynaecological surgery and childbirth may also provide the opportunity for *B. fragilis* to cause infection; postpartum endomyometritis is one example [40]. As a result of the risk from anaerobic infection, metronidazole is routinely administered as prophylaxis for patients undergoing gastrointestinal and gynaecological surgery. To date, metronidazole resistance has been relatively rare, but the development of multidrug-resistant *B. fragilis* (e.g. [6, 41]) is a potential cause for concern.

The classical course of *B. fragilis* infection resulting from intestinal damage initiates with gross contamination of the peritoneal cavity by the GI tract microbiota, which if not resolved by lymphatic clearance, progresses to a limited polymicrobial infection frequently composed of *Escherichia coli*, *Streptococcus* 'milleri' group and *B. fragilis*, with fibrin abscess formation and containment [39, 42]. If untreated, this may progress to chronic infection, monomicrobial *B. fragilis* abscesses at multiple body sites and potentially *B. fragilis* bacteraemia. In addition to intra-abdominal abscesses, *B. fragilis* is a common cause of liver, perianal, pilonidal and perineal abscesses [39, 43]. While *B. fragilis* is normally associated with infection below the waist, it can also cause, for example, lung and brain abscesses, particularly in individuals with other serious underlying conditions. As is the case for intra-abdominal abscesses, these infections are usually initially polymicrobial or alternatively seeded via bacteraemia [39].

Of the family *Bacteroidaceae* in the GI tract, *B. fragilis* is the most common isolate in the microbiology diagnostic laboratory and is highly clinically relevant [44]. It is the *Bacteroides* species associated with infection in 60% or more of clinical samples. *B. thetaiotaomicron* is the next most frequent, isolated from 17% or fewer of infections, while *B. ovatus* is isolated from fewer than 8% (reviewed in [1]). Multiple reports, including analysis of more than 3000 clinical specimens from the Wadsworth Anaerobe Collection database in the USA [2], confirm these data and the high association of *B. fragilis* with infection when compared to other *Bacteroidaceae*. It is not, however, the most numerous of the *Bacteroides* and related species detected by faecal culture. *B. thetaiotaomicron* and *Phocaeicola* (*Bacteroides*) *vulgatus* are reported to account for between 15–29 and 43–45%, respectively (reviewed in [1, 9]). The proposal to reassign *B. vulgatus* and 13 other former *Bacteroides* spp. to the genus *Phocaeicola* [45], but to retain this genus within the family *Bacteroidaceae*, is based on phylogenetic analyses. It should be noted that the type strain of *Phoaeicola*, *P. abscessus*, which was isolated from a brain abscess, is not bile-tolerant and is described as motile [46]. These characteristics were either dismissed or not considered in the proposal to include *B. vulgatus* in this genus. In the light of this, there may be further alterations to the taxonomic position of these bacteria in the future. Despite the numerical dominance of members of the *Bacterioidaceae* other than *B. fragilis* in the human colon, they are infrequently isolated from infection. Where they are isolated clinically, this usually represents gross faecal soiling of tissues with mixtures of anaerobic species [39]. Considering the complexity and diversity of the GI tract microbiota, it is intriguing that *B. fragilis* is the most frequently encountered Gram-negative anaerobic clinical isolate and a cause of serious and potentially life-threatening extra-intestinal infection. It appears, therefore, that *B. fragilis* has a greater pathogenic potential than other *Bacteroides* and related species of the gut microbiota. The question still to be answered is precisely why this is the case.

The multifactorial nature of bacterial virulence remains a challenge in determining the key characteristics necessary to **gen**erate the **patho**logical changes (pathogenicity) associated with infection, and *B. fragilis* is no exception. Although it lacks the characteristics

necessary to cause acute gastrointestinal infection, associated with classical enteric pathogens in relation to invasive disease, it clearly has characteristics that enable it to survive in the body outside the GI tract and overcome host defence mechanisms. Insights into the virulence of *B. fragilis* can be gained by considering the *B. fragilis* infectious process.

A taxonomic note

Two distinct lineages of *B. fragilis* have long been recognized, designated divisions I and II (the latter has more recently been designated *B fragilis* A in sequence databases). The initial defining feature of the two groups, which is of clinical treatment importance, relates to the type of beta-lactamase produced. Beta-lactamase gene carriage is considered to be universal in *B. fragilis*; division I carry the gene *cepA*, which encodes an active site serine beta-lactamase distantly related to Ambler class A, which is susceptible to inhibitory compounds such as clavulanic acid, sulbactam and tazobactam. Division II (*B. fragilis* A) carry the *cfiA* or *ccrA* gene, which encodes an Ambler class B zinc-requiring metallo-beta-lactamase and will therefore be resistant to beta-lactamase inhibitors when this beta-lactamase is expressed (reviewed in [1]). It should be noted that gene carriage may not reflect beta-lactamase expression; for example, insertion sequence activation is known to regulate gene expression [47]. Whole-genome sequencing identifies further genetic differences, which suggests that these two divisions could be defined as different species [48]. Distinguishing these lineages in diagnostic testing could inform choice of antibiotic. Any name change, however, would need careful consideration to retain clinical understanding that these two lineages may be similar with respect to disease and potential disease progression, as from a clinical diagnostic perspective these are isolated from the same types of infection.

Virulence

There has been much interest in the *B. fragilis* enterotoxin (BFT), particularly in relation to colorectal cancer and the potential for GI epithelial damage [12]. This damage could also impact on abnormal GI tract pathologies, such as inflammatory bowel and autoimmune conditions, by allowing localized movement of GI tract contents across the epithelial barrier [49]. BFT was first described as being associated with diarrhoeal disease in newborn lambs [50] and subsequently in other animals (e.g. [51]). It is recognized as a cause of diarrhoea in humans, in particular in children >1 year old (e.g. [52]), but asymptomatic carriage of BFT-positive *B. fragilis* may be as high as 40% [53], as determined in stool samples, and in mucosal tissue samples 50–55% [12]. The enterotoxin gene is frequently lacking in clinical isolates, including the type strain NCTC 9343. For example, it was only detected in 12 of 65 clinical isolates from a range of geographical regions [54]. In another study only 19% of blood culture isolates were positive for carriage of BFT [55]. BFT is not an intracellularly acting toxin, it is a zinc-dependent metalloprotease, thought to disrupt the integrity of GI epithelium by binding to an epithelial cell receptor and initiating E-cadherin cleavage [12]. The growing evidence for the association of BFT with colorectal cancer is compelling. A comprehensive review of the potential role of BFT in human disease can be found in Ref. [49]. While BFT, along with other peptidases, may contribute to virulence, with some indication of potential involvement in mouse models of sepsis [56], it is clear that the enterotoxin alone is unlikely to be an essential driver of *B. fragilis* infection, given its absence from so many clinical isolates. Indeed, host interaction with surface polysaccharides may be of importance in lethal sepsis [57]. It is likely, however, that *B. fragilis* proteolytic activity, including production of papain-like proteases [58], does contribute to virulence.

Iron acquisition is a key bacterial virulence determinant. As most bacteria have an absolute requirement for iron, sequestration of iron is an important first-line host immune defence mechanism, sometimes referred to as nutritional immunity. *B. fragilis* has the ability to overcome nutritional immunity and obtain iron within the host via multiple mechanisms. For example, genetic disruption of the ferrous transport fusion protein-encoding gene (*feoAB*) prevented growth and survival in a peritoneal infection model, indicating that inorganic iron uptake is essential for *in vivo* survival and therefore a central virulence determinant [59].

Clearly the ability of *B. fragilis* to utilize host components as substrates for growth, as detailed above in the context of the microbiome, is transferrable to survival outwith the GI tract. Indeed, sialidase homologues evident in *B. fragilis* genomes and sialidase activity were initially investigated in the context of virulence [60]. Sialidases (or neuraminidases), which cleave sialic acid from oligosaccharides on host cell glycoproteins or glycolipids, are recognized as virulence determinants in a number of pathogens [61] and may contribute to the pathogenic potential *B. fragilis*.

Although *B. fragilis* is defined as an obligately anaerobic bacterium, and requires anaerobic conditions to thrive and for successful isolation from clinical samples, it must survive exposure to oxygen to enable GI colonization of neonates [13]. There is evidence that *B. fragilis* is more aerotolerant than other *Bacteroides* species, enabling it to persist in, for example, the peritoneal cavity until the growth of facultative bacteria provides a sufficiently low redox environment for growth. Optimal isolation from clinical samples requires minimal exposure to oxygen and a redox potential >−42 mv at pH 7, at which point resazurin indicator dye will be colourless [62]. A separate issue is the toxicity of reactive oxygen. Production of enzymes such as superoxide dismutase and catalase play a role in detoxification [63], with variation in production amongst species and isolates possibly related to virulence (reviewed in [1]).

The extensive surface structure variation observed in *B. fragilis* is likely a key adaptation to changing nutritional conditions and bacteriophage interaction within the GI tract. It is also likely to aid immune system avoidance during an infection. The study of *B.*

fragilis surface components and molecular interactions, however, presents a major challenge. A single clonal isolate can produce 10 or more antigenically and potentially chemically different surface polysaccharides, each from a different biosynthetic operon spaced around the chromosome (Fig. 1). The surface structures formed include a large capsule and a small capsule, detectable by negative staining and light microscopy and multiple antigenically different microcapsules detectable as an electron-dense layer adjacent to the outer membrane by electron microscopy. *B. fragilis* does not produce a classical enteric O-antigen [1, 64].

An individual cell can simultaneously carry more than one type of capsule and the surface polysaccharides present on outer membrane vesicles released from the bacterial cells can differ from those on the cell surface [65, 66]. Production varies as the bacterial clone grows and divides with reversible ON/OFF switching of individual operons controlled by DNA inversion of upstream promoter regions (Fig. 1) [23, 67] and transcriptional termination regulation [68]. In addition, different clonal isolates can have antigenically different 'sets' of multiple polysaccharide biosynthesis operons, with more than 30 different operons identified across different clonal isolates [69]. Research focused on the zwitterionic microcapsule polysaccharide designated PSA produced by the *B. fragilis* type strain NCTC 9343 indicates a key role in abscess formation and T-cell interaction [24, 70]. *B. fragilis* NCTC 9343 PSA has been shown to have both pro- and anti-inflammatory activities [24]. The diversity of surface polysaccharides amongst and between different isolates, coupled to within-clone variable production, presents a problem when resolving the role of an individual polysaccharide in *B. fragilis*.

Dissemination of infection is likely to involve the inhibition of abscess formation and abscess rupture. Fibrinogen, a key component of the blood clotting system, forms the thick fibrin wall of an abscess. *B. fragilis* produces a human fibrinogen-binding protein and multiple fibrinogen-degrading proteases enzymes. These, in combination, could reduce or inhibit abscess formation and promote dissemination of infection and bacteraemia [71]. Further virulence characteristics include an ability to avoid the host's immune defences, including phagocytic uptake and killing and complement mediated killing (reviewed in [1]), although to date the molecular detail of these interactions remains to be determined.

Clearly characteristics of *B. fragilis* that have developed under the selective pressures in the GI tract microbiome are transferrable to survival in human host environments where the immune system has evolved to actively prevent bacterial survival.

CHALLENGES

The major challenge in the study of *B. fragilis* is the combination of strain diversity and within-strain reversibly variable expression of components, including surface structures. Operon divergence over evolutionary time and horizontal gene transfer combined have resulted in a formidable *B. fragilis* pan-genome that contains a large pool of diverse polysaccharide biosynthesis loci and multiple DNA restriction and modification systems and polysaccharide utilization loci. Horizontal gene transfer is driven by multiple integrative and conjugative elements, including conjugative transposons, integrated phage, mobilizable transposons and plasmids [1]. In addition, large-scale chromosomal transfer and integration [72] is a major driver of strain diversity, contributes to dissemination of the pan-genome and will aid rapid adaptation to changing environments. The multiplicity of DNA inversion events, driving within-strain variable expression, presents an additional challenge to the study of *B. fragilis*, not least in relation to genome assembly; extracted DNA contains sequences with inversions in different orientations (Fig. 1) [23]. Inversion events occur at a rate such that genes and operons switch off and on reversibly during culture. Physiological and biochemical characteristics will therefore not be consistent within a population or on subculture, even from a single colony [73]. The individual role in health and disease of each of the multiple antigenically different *B. fragilis* surface polysaccharides encoded in the *B. fragilis* pangenome therefore remains an unsolved puzzle. Forensic analyses based on precise genomic annotation and targeted mutation to fix DNA inversions in single orientations will be necessary to begin to fully understand the complex interactions and adaptations of *B. fragilis* both as a benign or sometimes harmful resident in the GI tract microbiome and as a pathogen during extraintestinal infection.

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Conflicts of interest
The author declares that there are no conflicts of interest.

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