Title: A Review of Segmented Filamentous Bacteria – Commensal Microbes with Potential Impact on Research

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Abbreviations: SFB, segmented filamentous bacteria; TLR5, Toll-like receptor 5
Abstract

Segmented filamentous bacteria (SFB) are commensal bacteria first identified in the ileum of mice and rats. Morphologically similar bacteria have been reported in a broad range of host species, however all strains have been refractory to in vitro culture thus far. While SFB were once considered innocuous members of the intestinal microbiota of laboratory rodents, they have now been shown to impact the development of the immune system in rodents and, subsequently, the phenotype of models of both enteric and extraintestinal disease. Thus, SFB represent long-recognized commensal bacteria serving as an intercurrent variable in studies using rodent models of disease. Herein, we present the basic biology of SFB followed by a provocative discussion on the immunological and physiological effects of colonization with SFB, with particular attention to the impact on rodent models of disease. We also propose that SFB may represent the tip of the iceberg in our understanding of the influence of the microbiota on model phenotypes. As next-generation sequencing techniques are increasingly used to interrogate members of the microbiota refractory to culture, we are likely to identify other commensal microbes impacting the models we use. This review underscores the need to characterize such host-microbe interactions, as animal research represents a critical tool that is particularly vulnerable to scrutiny in an era of decreasing financial resources, and increasing accountability for the use of animal models.

History

Almost half of a century ago, a few astute microscopists reported a unique microbe which remained securely attached to the epithelium of the ileum in mice (Mus musculus) and rats (Rattus norvegicus) despite removal of other luminal contents\(^{32,69,71,72}\). First postulated to be a fungus based on its size and unusual morphology\(^{69}\), electron microscopy soon revealed the microbe to be a segmented bacterium containing “round forms”, some of which appeared to be in the process of dividing. Additionally, one end of the microbe appeared to be specialized for integration among and attachment to the epithelial brush border. A very similar organism was soon reported in the ileum of chickens (Gallus domesticus)\(^{25}\) and dogs (Canis familiaris)\(^{12}\), although the relation of microbes from the various hosts remained unclear. In 1974, Davis and Savage published the first report of the habitat, ultrastructural morphology, and proposed life cycle of what is now commonly referred to as segmented filamentous bacteria (SFB)\(^{13}\). There is abundant anecdote among users of animal models regarding the loss or alteration of model phenotypes when using animals purchased from different vendors, or when housing animals under different husbandry conditions. These changes have often been attributed to genetic drift of rodent
strains maintained at different institutions, or to unknown factors of the host microbiota. Recent studies comparing mice from different vendors\textsuperscript{19, 37, 38} have identified SFB as one of those pivotal members of the commensal microbiota with significant effects on the ontogeny and function of the host immune system. Accordingly, what was once an incidental finding on histological examination of the gastrointestinal tract should now be considered a variable with the potential to affect outcomes in several disease models. Identification of affected models and the extent to which they are impacted is a critical need in biomedical research.

**Unique morphology**

SFB are Gram-positive, spore-forming, filamentous bacteria ranging between 0.7 and 1.8 µm in diameter and up to 80 µm in length\textsuperscript{10}, that selectively colonize the ileum of mice and rats shortly before weaning\textsuperscript{43, 71} (Figure 1). The first segment of the microbe possesses a nipple-like appendage, called a holdfast, that projects into the plasma membrane of the enterocyte, without actually rupturing or penetrating the host cell wall\textsuperscript{4, 10, 13, 21, 79} (Figure 2). It should be noted however that attachment of SFB does induce focal displacement of the microvillar brush border and alterations in the electron density of the enterocyte plasma membrane and apical cytoplasm. Jepson \textit{et al.} documented actin polymerization at the apical surface of enterocytes at the site of attachment\textsuperscript{40}, suggesting pedestal formation, an active host cellular response similar to that induced by invasive or adherent pathogens such as \textit{Escherichia coli}\textsuperscript{22} and \textit{Salmonella typhimurium}\textsuperscript{23}. The remainder of the microbe is septate, with each mature segment of the body containing between zero and two, but typically one, intrasegmental body, originally thought to be spores.

**Presumed life cycle and transmission**

Chase and Erlandsen observed that there are actually two morphologically distinct types of intrasegmental bodies which are either developing holdfasts or spores, suggesting that SFB exist in both a vegetative and dormant form\textsuperscript{10}. Both types of intrasegmental body appear to increase in maturity in the proximal to distal direction and functional holdfasts or spores are thought to be released from the mature distal segments of the microbe. It should be remembered however that much of the knowledge of the life cycle of SFB has been deduced from microscopy and, as all attempts to culture the organism have been fruitless\textsuperscript{14, 46}, there are still many uncertainties. For example, one can infer that SFB must complete a full life cycle within two to three days given the rapid turnover of intestinal epithelial cells in rodents. Similarly, as SFB are considered obligate anaerobes and spores are seen free in the lumen of
infected rodents\textsuperscript{69}, their spread is presumably via inoculation with spores. While colonization by SFB is affected by several factors including diet and the immune status of both the dam and pup\textsuperscript{43, 47, 52}, once SFB are introduced into a colony, they are transmitted vertically and have the potential to become endemic\textsuperscript{13}. Longitudinal studies of the developing ileum have confirmed that SFB appears in juvenile mice at around 20 days of age and that, during the earliest stage of colonization, the SFB are themselves transiently colonized by other rod-shaped bacteria\textsuperscript{51}. Soon thereafter, SFB proliferate to the point that they are one of the dominant bacterial genera present in the gut (Figure 3) before receding to the levels seen in adults\textsuperscript{78}. Considering the above, the first few weeks after weaning may represent the optimal testing window for the determination of SFB status in a mouse colony. Current diagnostic modalities include histological examination of the ileum and PCR-based methods. Histological examination requires an experienced pathologist to differentiate SFB from the myriad smaller filamentous microbes in the gastrointestinal tract, and is a post-mortem assay. Alternatively, multiple primer sets designed against the 16S rRNA gene have been published\textsuperscript{24, 77} allowing for noninvasive screening of feces from rodents. This can easily be performed in-house and is also commercially available for rats and mice.

**Host specificity**

There is now evidence that SFB-like bacteria exist in a broad range of species including rhesus macaques (\textit{Macaca mulatta})\textsuperscript{48}, crab-eating macaques (\textit{Macaca fascicularis})\textsuperscript{48}, vervet monkeys (\textit{Cercopithecine aethiops})\textsuperscript{6}, African gorillas (\textit{Gorilla sp.})\textsuperscript{67}, South African claw-footed toads (\textit{Xenopus laevis})\textsuperscript{48}, carp (\textit{Cyprinus carpio})\textsuperscript{48}, rainbow trout (\textit{Oncorhynchus mykiss})\textsuperscript{17, 92}, wood mice (\textit{Apodemus sylvaticus})\textsuperscript{48}, guinea pigs (\textit{Cavia porcellus})\textsuperscript{46}, rabbits (\textit{Oryctolagus cuniculus})\textsuperscript{33}, horses (\textit{Equus caballus})\textsuperscript{56}, cattle (\textit{Bos taurus})\textsuperscript{75}, pigs (\textit{Sus scrofa domestica})\textsuperscript{70}, cats (\textit{Felis catus})\textsuperscript{48}, turkeys (\textit{Meleagris gallopavo})\textsuperscript{15, 68}, jackdaws (\textit{Corvus monedula})\textsuperscript{48}, and magpies (\textit{Pica pica})\textsuperscript{48}. Perhaps not surprisingly, SFB have also been reported in humans although there are conflicting data regarding its persistent colonization of adults. In the first published report of SFB in humans, SFB were visualized via light microscopy adherent to biopsied tissue from one of six adults examined\textsuperscript{48}. Conversely, a recent attempt to confirm the presence of SFB in humans by searching for any part of the SFB genome in 263 human metagenomic data sets was unsuccessful. Similarly, attempts to detect SFB using PCR designed to amplify five different SFB-specific genes from eight freshly evacuated human fecal samples were also fruitless\textsuperscript{73}. These divergent findings may be rectified by a recent study wherein SFB were found to colonize humans in an age-dependent manner. SFB were detected by PCR in 25% (2/8) of infants under 6 months of age and 78.6% (11/14) of infants between 7 and 12 months of age\textsuperscript{95}. However, the same study reported a prevalence of only
6.2% (10/162) in individuals between the ages of 3 and 75 years. Thus, it appears that SFB commonly colonize the gut of humans early in life but are then cleared in most individuals by three years of age. An age-dependent decrease in SFB colonization may occur in other species (dogs, chickens, horses, and pigs) as well. Also of interest is a recent retrospective histological survey of ileo-cecal valves of patients diagnosed with ulcerative colitis or Crohn’s disease (the two primary forms of inflammatory bowel disease), and patients without a history of intestinal inflammation. 100% (6/6) ulcerative colitis patients were colonized by high levels of SFB, while SFB was found in none (0/6) of the Crohn’s disease patients, and only low levels in 50% (3/6) of the control samples. While these results are purely correlative, the relationship between SFB and inflammatory bowel disease or other conditions in humans merits further investigation.

SFB selectively colonize the ileum of all species examined (with the exception of rainbow trout and carp which lack well-differentiated ilea, and the chicken in which SFB also colonize the cecal tonsils). Being highly dependent on other organisms for nutrients, it is tempting to speculate that SFB colonize the gut in such a site-specific manner due to a relatively greater availability of a particular host-derived factor, such as cobalamin (vitamin B12), in that region. Alternatively, the ileum is also the region of the gut wherein bacterial densities increase dramatically, potentially providing some essential microbially produced factor. There are also species-specific differences in the predominant site of SFB attachment within the ileum. In pigs and most rodents, SFB adhere to both absorptive villi and the follicle-associated epithelium overlying Peyer’s patches; in mice and horses however there is a reported preference for attachment to the follicle-associated epithelium. Alternatively, microscopic examination of ilea from dogs, cows, and rabbits revealed SFB attached primarily to the absorptive villi. At the cellular level, SFB appear to be capable of binding to the apical surface of classic absorptive enterocytes, specialized M cells, and goblet cells, as well as to the tight junctions between these cells. Additionally, Meyerholz et al. documented SFB in direct contact with intraepithelial mononuclear cells. The portion of the filament in contact with the host cell appeared degenerate leading to the speculation that the microbe was in an early stage of phagocytosis, a process which has been noted elsewhere.

The first piece of evidence that SFB exhibit host specificity came from experiments wherein germ-free rats and mice were gavaged with ileal homogenates from both species. Tannock et al. demonstrated that SFB could subsequently be found in only those animals that had received the ileal microbiota derived from its cognate host species. Studies performed with mice and chickens reached a similar
conclusion, yet the phylogenetic relation of these host-specific SFB to each other and to other microbes remained unclear. A breakthrough in the study of SFB occurred with the successful mono-association of germ-free mice via intraileal inoculation with ethanol-treated ileal contents of donor mice. In the absence of an effective method of culture, this provided a pure sample for sequencing of the 16S rRNA gene in mouse SFB. Comparison of the 16S rRNA sequence of mouse SFB to metagenomic data from rats, chickens, and macaques revealed that the microbes belong to a distinct group within the phylum Firmicutes, putatively serving as a novel genus in the order Clostridiales. Sequencing of the complete genome of rat and mouse SFB has confirmed that they are indeed closely related to, but distinct from, recognized Clostridium spp. Additionally, comparison of 16S rRNA sequences of SFB isolated from mice and rats to existing sequences generated from fecal samples of multiple diverse species including gorilla, macaque, dog, and rainbow trout demonstrated 94-98% nucleotide identity, forming a distinct cluster separate from other Clostridium spp. (Figure 4). It should be noted that while SFB has also been reported in a number of invertebrate species including myriapods, termites, cockroaches, isopods, and beetles, 16S rRNA analysis has placed those microbes in the family Lachnospiraceae, rather than Clostridiaceae, and thus distinct from the SFB found in vertebrate species. Similarly, reports of long segmented filamentous organisms associated with visceral granulomatous disease, stunting syndrome, diarrhea, and increased mortality in chickens, turkeys, and quail, must be evaluated cautiously. Considering the presence of these long segmented filamentous organisms in the jejunum, their occasional branching morphology, and their association with overt disease, it is more likely that these are microbes distinct from SFB such as perhaps Actinomycetes spp. or Nocardia spp. The fact that microbes more akin to the SFB characterized in rodents have been identified in the cecum of healthy turkey poults makes this interpretation even more likely. Regardless, molecular techniques will need to be employed to definitively answer this question.

**Genome and molecular biology**

Considering the mutualism implied by the close association of SFB with the ileal enterocytes and lack of inflammation, Davis and Savage posited that SFB coevolved with mice and rats and that the microbe may rely on the host for nutrients. Providing strong support for that insight, three groups have now independently sequenced the complete genome of mouse SFB with one of them also sequencing the genome of rat SFB. Additionally, a fourth group has performed single cell sequencing of five individual SFB filaments. The genome of mouse SFB encodes a single circular chromosome of between 1.57 and 1.62 Mb with a G+C content of approximately 28%, similar to that found in other obligate
symbionts. SFB possess a remarkably reduced genome relative to closely related *Clostridium* spp., and lack genes responsible for the synthesis of the majority of amino acids, nucleotides, vitamins, and cofactors. Conversely, several transporter and permease genes, necessary for the uptake of extracellular nutrients, are found in the genomes of both rat and mouse SFB. It is also likely that SFB facilitate the acquisition of nutrients through the degradation of host and dietary proteins; the rat SFB genome contains putative genes for 28 proteases and 53 peptidases, several of which are likely secreted\cite{67}.

Expectedly, there are also many genes involved in sporulation and germination homologous to those found in clostridial species, although the triggers for these processes are unknown. While exposure to oxygen is presumably one cause for sporulation, the fact that spores are constitutively released in the ileum\cite{69,75,76,77} suggests that sporulation may be an ongoing process in the life cycle of SFB. Additionally, the presence of genes encoding peroxidase and catalase proteins implies that SFB may be able to survive in microaerophilic environments.

The presence of multiple coding DNA sequences of bacteriophage origin in the genome of both rat and mouse SFB imply that SFB are subject to foreign invading DNA. Some of these coding DNA sequences are highly similar to those found in *Clostridium* spp. suggesting horizontal gene transfer may have occurred. Additionally, the genome of both mouse and rat SFB contain clustered, regularly interspaced, and short palindromic repeat (CRISPR) loci, which function as acquired components of prokaryotic immune systems. CRISPR loci are found in a large percentage of sequenced bacteria and archaea and are thought to serve as an indicator of past exposure to invading DNA\cite{3,60}.

One of the most fascinating findings in the genome of rat and mouse SFB is the presence of genes encoding multiple flagellar assembly proteins, including four different flagellin loci in rat SFB, and three\cite{67} or four\cite{54} in mouse SFB. While no evidence of SFB motility has ever been documented, the presence of a complete set of chemotaxis genes and an absence of recognizable pseudogenes in the flagellin gene sets suggest that SFB may possess chemotactically driven flagellar motility\cite{54}. That being said, rat SFB also possess genes encoding several type IV pilus proteins\cite{67} previously shown to be involved in a twitching motility\cite{61}. Additionally, flagellar assembly proteins may be employed for uses other than motility such as type three secretion systems or as adhesins\cite{73}. Thus, the exact method of motility used by SFB remains a mystery although the presence of multiple genes involved in chemotaxis, along with their ability to penetrate the mucus layer lining the intestinal epithelium, suggest that they are, at least transiently, motile.

**Impact on host physiology**
SFB has historically been considered a member of the commensal microbiota. However, it is now becoming very clear that its presence can have a profound influence on models of intestinal disease as well as systemic immune-mediated diseases. How often has the laboratory animal veterinarian heard the comment, “my model worked fine at my old institution”? Could such anecdotes of altered model phenotypes be associated with subtle changes in commensal microbiota such as the addition of SFB?

The following paragraphs highlight the current knowledge regarding the influence of SFB on the development and homeostasis of several components of the mucosal immune system. As our understanding of SFB is still in its infancy, the reader is encouraged to regularly review the literature for new information that is likely to emerge about this bacterium, as well as other members of the commensal microbiota.

Interest in a functional role of SFB in host health was initially spurred by evidence suggesting that SFB contributed to colonization resistance to the enteric pathogen *Salmonella enteritidis*\(^{27}\). Garland *et al.* showed that in rats experimentally infected with a virulent strain of *S. enteritidis*, the presence of SFB and Salmonella on the ileal epithelium of individual villi is mutually exclusive, implying that SFB not only physically compete with pathogenic microbes for binding spots on enterocytes, but that they also induce a local response which hinders the ability of *S. enteritidis* to adhere to the epithelium. Similarly, the presence of SFB on the ileal villi of rabbits correlates with resistance to enteropathogenic *Escherichia coli*, another microbe known to enter the host through ileal Peyer’s patches\(^{33}\). Using mice in which the microbiota differed by only the presence or absence of SFB, Ivanov *et al.* showed that SFB increases resistance to colonization by another microbe of the family *Enterobacteriaceae*, *Citrobacter rodentium*\(^{37}\).

Notably, the influence of SFB on colonization resistance likely requires the presence of other commensal microbes as SFB mono-associated mice fare no better than germ-free mice when challenged with *S. enterica* serovar Typhimurium\(^{11}\).

**Impact on host immune system**

With the possible exception of rainbow trout\(^{15-18}\), no host has ever been documented to show an inflammatory response, either grossly or microscopically, to naturally occurring colonization with SFB. That being said, SFB is clearly not an idle bystander in its interactions with the complex mucosal immune system. Mice mono-associated with SFB possess significantly higher numbers of intestinal IgA-secreting cells than germ-free mice, and significantly higher IgA titers in the intestines and serum. Remarkably, mono-association of germ-free mice with SFB restores the production of IgA to levels close to those seen in SFB-negative specific pathogen-free mice\(^{50, 91}\). Talham *et al.* extended these findings to show
that SFB induce not only specific IgA production but also “natural” non-specific IgA production, with SFB-specific IgA production comprising less than 1.4% of total IgA. Conversely, maternal IgA production has been shown to inhibit SFB colonization of pups during suckling, perhaps explaining early reports in conventionally housed mice that SFB is not evident in pups until just before weaning. SFB undergo robust proliferation after weaning but then decline to a basal level shortly thereafter, presumably due to host-derived IgA. The presence of IgA apparently impacts the proliferation of SFB in adults also as mice lacking activation-induced cytidine deaminase, an enzyme critical in hypermutation of IgA, experience a selective overgrowth of SFB despite the presence of other commensal organisms.

SFB also influence the development of the T cell repertoire. SFB enhance the development of activated (CD45RBlow) CD4+ T helper cells in Peyer’s patches and the expansion of both ααTCR- and αβ TCR-bearing intraepithelial lymphocytes. In stark contrast to experiments performed with mice mono-associated with various members of the dominant bacterial phyla in the gut including Bacteroides thetaiotaomicron, B. vulgatus (phylum Bacteroidetes), three different Clostridium spp. from the Altered Schaedler Flora (phylum Firmicutes), or E. coli (phylum Proteobacteria), mono-association with SFB recapitulates many of the immunological effects of a complex microbiota. To this end, SFB induce a full retinue of homeostatic CD4+ T helper cell profiles including Th1, Th2, Th17, and TREG cells, with the most pronounced effect being on the Th17 type. The Th17 response induced by SFB is linked to the induction of anti-microbial responses and increased colonization resistance to pathogenic Enterobacteriaceae. This is likely due to the protective effects of IL-22, a canonical Th17 cytokine known to induce the production of antimicrobial peptides efficacious against bacteria capable of inducing attaching and effacing lesions such as C. rodentium and certain strains of E. coli.

The presence of flagellar proteins may explain the ability of SFB to induce IgA production, as well as Th17 immune responses. Kuwahara et al. showed that recombinant gene fusion proteins of three of the four mouse SFB flagellins activated the NF-κB signaling pathway in a Toll-like receptor 5 (TLR5)-dependent manner. TLR5 is a pattern recognition receptor of the innate immune system expressed primarily on the CD11c+CD11b+ subset of intestinal dendritic cells, and binding of flagellin has been shown to induce class-switching to IgA and the differentiation of Th17 cells. Of note, Denning et al. demonstrated that this subset of dendritic cells was solely responsible for the differentially greater production of IL-17 in the gut of C57BL/6 mice harboring SFB (from Charles River Laboratories) relative to C57BL/6 mice lacking SFB (from The Jackson Laboratory). The binding sites of flagellin and TLR5 have been identified and, notably, the specific motif capable of binding TLR5 is highly conserved in the flagellar
proteins of SFB while this motif is absent in all commensal *Clostridium* spp. examined except one, *C. sporogenes*. Thus, it seems plausible that SFB invoke the production of IgA and the differentiation and proliferation of Th17 cells through the sensing of SFB-specific flagellin proteins by TLR5 expressed by the CD11c<sup>hi</sup>CD11b<sup>hi</sup> subset of intestinal dendritic cells.

In addition to adaptive immunity, colonization with SFB also influences the innate immune system. Colonization of germ-free mice with SFB, but not a mixture of *Clostridium* spp., leads to increased expression of MHC class II molecules on intestinal epithelial cells<sup>30, 91</sup>. SFB also influence the glycosylation of enterocytes, specifically inducing the expression of fucosyl asialo GM1 glycolipids, posited to inhibit the attachment of other adherent microbes<sup>30, 90</sup>. Additionally, Keilbaugh *et al.* demonstrated that SFB contribute to innate immunity via enhanced production of IFN-γ by NK cells, and the induction of RegIIIβ and RegIIIγ on intestinal epithelium<sup>45</sup>, a result duplicated by Ivanov *et al.*<sup>37</sup>.

**Impact on animal models**

As our understanding of the role of SFB in the maturation of the immune system has grown, several researchers have applied SFB as an experimental variable to assess its impact on inflammatory disease models. In one commonly used model of inflammatory bowel disease, CD4<sup>+</sup>CD45RB<sup>high</sup> effector subsets of T cells isolated from the spleen of conventional mice are adoptively transferred to severe combined immunodeficiency mice to induce severe colitis while co-transfer of CD4<sup>+</sup>CD45RB<sup>low</sup> cells prevents the development of inflammation<sup>66</sup>. As in most models of inflammatory bowel disease, the development of inflammation is dependent on the presence of intestinal microbiota. Efforts to determine the minimal bacterial community needed to allow for inflammation demonstrated that severe combined immunodeficiency mice mono-associated with SFB do not develop colitis upon transfer of CD4<sup>+</sup>CD45RB<sup>high</sup> T cells<sup>54</sup>, while severe combined immunodeficiency mice colonized with a very limited defined (specific pathogen-free) microbiota develop mild to moderate inflammation by twelve weeks post-transfer<sup>80</sup>. Interestingly, the addition of SFB to the specific pathogen-free microbiota consistently results in severe colitis in recipient mice, suggesting that SFB exert a synergistic effect with other commensal bacteria in providing an environment conducive to intestinal inflammation<sup>80</sup>.

Collectively, the above findings highlight the importance of SFB in the development and modulation of several components of the mucosal immune system including innate, humoral, and cell-mediated components. Given the importance of mucosal immunity to virtually all models of intestinal disease, the fact that SFB has such profound influence on this system raises many questions regarding its impact on
models of intestinal disease. Moreover, there is growing evidence that the mucosal immune system has key interactions with the development and homeostasis of systemic immunity. Thus the breadth of models potentially altered by the presence or absence of this bacterium is greatly expanded. For example, other groups have now shown that SFB exacerbates inflammation in models of extraintestinal disease as well, including experimental autoimmune encephalomyelitis\textsuperscript{55} and autoimmune arthritis\textsuperscript{93}, in a T\textsubscript{H}17-dependent manner. Interestingly, SFB appear to confer protection from diabetes in nonobese diabetic mice, and in a sex-dependent manner\textsuperscript{57}, raising another potentially crucial set of questions regarding differential impact of SFB, and perhaps other microbes, on males and females.

**Implications for other commensal microbes**

The growing evidence that SFB can modulate mucosal health and subsequently alter rodent model phenotypes raises the question, “what about other members of the microbiota?” Clearly, SFB are not the first autochthonous bacteria to be identified as having an impact on disease models. *Helicobacter* spp., still prevalent in many research colonies but often unreported in the literature, can be considered commensal microbes in some strains of mice, but may also be necessary components for the phenotype of certain disease models\textsuperscript{59}. As with SFB, *Helicobacter* spp. may also influence host physiology, such as to affect immune responses to other commensal bacteria present in the gut\textsuperscript{41,42}. Similarly, several conceptual models have been proposed wherein key microbes modulate the composition of the microbiota so as to increase its overall inflammatory potential\textsuperscript{31,74,87}. Thus, the widespread influence of certain microbes on other members of the microbiota as well as the host, coupled with the fact that the majority of the intestinal microbiota is resistant to culture in vitro, make it highly likely that other gut microbes with unappreciated impact on disease models will be identified in the future. Additionally, studies of the human microbiota in individuals affected with various diseases often find correlations between disease and shifts in the relative abundance of higher taxa, such as differences in the ratio of *Firmicutes* to *Bacteroidetes*. While many of these findings are correlative, they beg the question: is there a core microbiota in rodents, necessary for normal mucosal immunity? And if so, what are the functions of that microbiota and the effect of subtle alterations in its composition? We have already begun a paradigm shift in our analyses of the microbiota, through next-generation sequencing technologies and bioinformatics-based approaches. These and other inter-disciplinary methods will be needed for more inclusive and robust assessments of the microbiota and the identification of other commensal microbes impacting animal models of disease.

**Conclusions**
While the existence of SFB has been recognized for several decades, it has only entered the forefront of microbial and metagenomic research in the last several years. Having carried multiple erstwhile names (bacillus of Savage, Candidatus Arthromitus, and now Candidatus Savagella\textsuperscript{62}), it has rightly attracted attention due to its role as a “type species” with the capacity to induce the development of multiple adaptive immune responses in the gut. For microbiologists and immunologists, SFB provide a unique model organism for investigation of the development of the immune system and host-microbe interactions. For any scientist utilizing mice or rats in their research, SFB must be considered as a potential variable with a potential impact on outcomes. Similarly, SFB is but one organism within a complex and dynamic mixture of microbes and the entire commensal microbiota needs to be considered when model phenotypes are altered or completely lost, particularly when changes in diet or environment are present. It must be remembered that only a minority of intestinal bacteria are amenable to culture\textsuperscript{20}, and everything known about SFB stems from those first observations of a bacteria with unique morphology and size. Doubtless, molecular metagenomic approaches will eventually identify other microbes with profound effects on host immunology and physiology, offering new insights into human and animal health.


Figure 1. Photomicrographs of a hematoxylin and eosin-stained section of segmented filamentous bacteria (SFB) in the ileum of a weanling (approximately 3 to 4 week old) female C57BL/6 mouse. Images captured at 200 × (A) or 1000 × (B) magnification.

Figure 2. Transmission electron microscopy images demonstrating the holdfast of a segmented filamentous bacterium (SFB) attached to the ileal epithelium of a weanling (approximately 3 to 4 week old) female C57BL/6 mouse. Images captured at approximately 27000 × (A) or 90000 × (B) magnification.

Figure 3. Scanning electron microscopy images of segmented filamentous bacteria (SFB) in the ileum of a weanling (approximately 3 to 4 week old) female C57BL/6 mouse. Images captured at 1500 × (A), 4500 × (B), or 35000 × (C) magnification.

Figure 4. Phylogenetic tree based on 16S rRNA sequences of segmented filamentous bacteria (SFB); The 16S sequences from mouse and rat SFB and three additional sequences identified in Prakash et al.; three published sequences from chicken, rainbow trout, and monkey SFB; and 28 Clostridium strains were used. A distinct clade composed of 16S sequences derived from the eight hosts, including mouse and rat, is boxed. Reprinted from Cell Host & Microbe, Vol. 10 (3), Prakash, Oshima, Morita, Fukuda, Imaoka, Kumar, Sharma, Kim, Takahashi, Saitou, Taylor, Ohno, Umesaki, and Hattori. Complete genome sequences of rat and mouse segmented filamentous bacteria, a potent inducer of Th17 cell differentiation, 273-284, Copyright (2011), with permission from Elsevier.