Trends in Microbiology

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Opinion

Bridging Bacteria and the Gut: Functional Aspects of Type IV Pili

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Cell-surface-located proteinaceous appendages, such as flagella and fimbriae or pili, are ubiquitous in bacterial communities. Here, we focus on conserved type IV pili (T4P) produced by bacteria in the intestinal tract, one of the most densely populated human ecosystems. Computational analysis revealed that approximately 30% of known intestinal bacteria are predicted to produce T4P. To rationalize how T4P allow intestinal bacteria to interact with their environment, other microbiota members, and host cells, we review their established role in gut commensals and pathogens with respect to adherence, motility, and biofilm formation, as well as protein secretion and DNA uptake. This work indicates that T4P are widely spread among the known members of the intestinal microbiota and that their contribution to human health might be underestimated.

Highlights

Pili are key interaction molecules in the context of the gut microbiota.

Type IV pili (T4P) are ubiquitous in bacteria and are of great functional importance.

About 30%, and potentially even up to 45%, of microbiota members are thought to express T4P, illustrating their importance in the context of microbiota–host interactions.

Functions are diverse and include (but are not limited to): adhesion, biofilm formation, motility, and molecule exchange [e.g., double stranded (ds)DNA and proteins].

T4P are found in all monoderm and diderm bacteria and in members of the Archaea.

Bacterial Outreach: Pili as Key Functional Appendages

Microbial interactions are essential for growth and survival. These include interactions of microbes with the abiotic environment as well as with other cells, including other microbes, viruses, and eukaryotic cells. In many cases these interactions are promoted by cell-surface-located structures that form protruding appendages, such as **flagella** and **pili** or **fimbriae** (see Glossary). Pili are proteinaceous hair-like appendages that offer bacteria a wide range of functional adaptations. Especially in the human gut, which is colonized by large bacterial communities, pili can be crucial for adhesion to host cells and molecules. The gut is an extremely versatile environment, characterized by constant turnover and flow as a result of food intake, mucin renewal, epithelial cell turnover, and peristalsis [1]. Previous deep metagenomic analyses of the gut microbiota have identified type I pili as a major factor enhancing survival and persistence of low-abundant species in the gut [2].

While microscopic observations were used for their initial identification, pili are presently classified based on their structure and mode of biogenesis. Here, we focus on **type IV pili** (**T4P**) since they are widely spread, functionally diverse, and share a similar genetic organization, protein composition (Box 1), and conserved biogenesis (Box 2) [3]. T4P are involved in adherence, DNA uptake, motility, biofilm formation, and protein secretion (Figure 1, Key Figure). Like many other surface appendages, T4P can also be functionally exploited by bacteriophages that use these pili as receptors [4]. T4P are typically 5–8 nanometers in diameter and can range up to several micrometers in length, exceeding the size of bacteria [5].

T4P are widely distributed in Gram-negative and Gram-positive bacteria, such as *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Vibrio cholerae*, and *Clostridium* sp., as well as in several Archaea [6–8]. Bacterial T4P are further divided into subtypes, namely **type IVa pili** (**T4aP**), **type IVb pili** (**T4bP**), and Tad pili, which recently have been designated as **type IVc pili** (**T4cP**) [3,9], which are all assembled using conserved mechanisms (see Figure I in Box 2). T4aP are characterized by the presence of a PilT retraction ATPase, resulting in retraction of the pilus which can generate large mechanical forces (nanoNewton range) [10]. This pilus retraction can result in motility

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Box 1. T4P Architecture

The most striking structural feature of T4P is the pilus, which primarily consists of major pilin subunits. This major pilin subunit is called PilA in the T4P model organism *P. aeruginosa*, and TcpA and BfpA in resp. *V. cholerae* and *E. coli*. [52]. The major pilins are synthesized as prepilins and targeted to the membrane by an αN-terminal sequence motif, the class III signal peptide. This positively charged signal peptide ensures proper orientation of PilA in the inner membrane [53]. Once in the inner membrane, the prepilin peptidase PilD cleaves off this leader sequence and methylates PilA (Box 2) [54]. After this, PilA can be incorporated into the growing pilus structure [54]. The major pilins of T4aP and T4bP are distinct, with the leader peptides of T4aP typically being less than ten amino acid residues in length and mature pilins ranging between 140 and 160 residues [9]. In T4bP, leader peptides are longer, namely 15–30 amino acids, and they precede a larger mature pilin of 180–200 residues [9]. The pilus is thus mainly built of PilA but can be further decorated with minor pilins. In bacteria expressing T4aP, a group of minor pilins, PilV, PilW, PilX, FimU, and PilE, are thought to form a priming complex for major pilin assembly (Box 2) [55]. Prepilin peptidase PilD is shown to process these minor pilins similarly to PilA, cleaving the leader sequence and methylating the pilin [56]. The minor pilins are thought to form a short stem that reduces the energy barrier for extension of the major pilin and can both be found at the tip and throughout the pilus [56]. Finally, T4P complexes can accommodate additional minor pilins and adhesins which confer extra specificity and functionality to the pilus – for example, ComP in *Neisseria* binds DNA [57], or PilY1, which aids in the adhesion of *P. aeruginosa* [55]. An overview of T4P biogenesis is given in Box 2.

(Box 2) [11]. However, *V. cholerae* T4aP have been shown to retract in the absence of a PilT retraction ATPase [12]. T4aP are widely spread and studied in detail in *P. aeruginosa* [13] and in *N. gonorrhoeae* [14]. T4bP are less prevalent and less uniform, and are produced by enteropathogenic *Escherichia coli* (EPEC) [15] and *V. cholerae* [toxin coregulated pili (TCP)] [16] amongst others**.** While most T4bP lack PilT, they can have another ATPase, for example, the BfpF ATPase of EPEC bundle-forming pili [17]. They can also use a different mechanism entirely, as in *V. cholerae* where TCP retraction is thought to be triggered by the incorporation of a specific minor pilin leading to spontaneous disassembly and retraction [18]. Tight-adherence (Tad) pili have smaller pilin subunits than other T4P and are encoded at a single genetic locus. They used to be considered a subclass of T4bP, but recent phylogenetic studies showed that they form a distinct phylogenetic clade, namely T4cP [3,19]. T4cP retraction also takes place in the absence of PilT. For example, in *Caulobacter crescentus*, T4cP retraction was shown to be regulated by CpaF, a bifunctional pilus motor that is also responsible for extension [20].

T4P Are Ubiquitous in the Gut Microbiome

Several omics studies and cultivation efforts have generated insights into species that are present in a healthy or disease-associated microbiota and allowed dedicated studies of its inhabitants

Box 2. Biogenesis of T4P

Biogenesis of T4P is strongly conserved, and bacteria expressing them harbor similar biogenesis genes, related to T2SS [44]. The first step in pilus assembly is the attachment of an ATPase, PilB (yellow, Figure I), to the cytoplasmic ring formed by PilM (blue) and platform protein PilC (green) [58] (Figure I). The cytoplasmic ring, PilM, forms together with subunits PilN (light gray), PilO (dark gray), and PilP (orange), an inner membrane alignment complex called PilMNOP [58] (Figure I). Attachment of PilB to PilM causes conformational changes in PilN and PilO, resulting in a cage-like ring in the inner membrane and periplasm [58,59]. This allows PilA (black) subunits to enter the complex, enabling pilus assembly [60]. The ATPase PilB is also attached to platform protein PilC, resulting in the incorporation of PilA subunits into the pilus, thus elongating the pilus [61]. Minor pilins are synthesized in a similar fashion to PilA and are thought to form a priming complex for major pilin assembly (cf. Box 1). They are mostly found at the pilus tip, but also along the length of the pilus [56]. In Gram-negative bacteria, the inner membrane PilMNOP complex needs an outer membrane pore to facilitate the transfer of the pilus through the periplasm during (de-)polymerization [62]. This outer membrane pore is formed by PilQ (red) which is linked to the inner alignment complex PilMNOP by PilP [58] (Figure I). PilQ has two internal gates, a secretin gate and a periplasmic gate, which are closed in the absence of a pilus to prevent leakage of molecules [63]. Obviously, PilQ is absent in Gram-positive bacteria where the pilus is directly sticking out through the peptidoglycan layer. Once fully assembled, the pilus can attach to a surface, thus inducing tension in the pilus. In T4aP, the retraction ATPase PilT (brown) is responsible for pilus retraction. The PilB ATPase can be released from the platform protein PilC, allowing PilT (brown) to interact with PilC [58], after which PilA subunits are released back into the membrane (Figure I) [14]. In T4bP this process occurs independently of the PilT ATPase [17,18].

Glossary

Biofilm: biofilms consist of bacteria protected by a matrix formed out of proteins, polysaccharides, DNA, and RNA. The first step in the formation of a biofilm is the attachment of bacteria to a surface. When several bacteria aggregate on a surface and protect themselves with a simple matrix, microcolonies are formed. Finally, when these aggregates continue to grow, and the extracellular matrix becomes thicker, biofilms are formed [1].
Conjugation: conjugation takes place when two bacteria connect to each other through type IV secretion systems to form a mating bridge through which DNA can be transferred [64].
Fimbriae: mostly used synonymously with pili [6,7].
Flagella: very long, slender appendages on bacteria, greatly varying in size and mostly implicated in motility. Rotation of flagella propels bacteria forward, leading to swimming motility in individual bacteria, or, when considering the whole colony, swarming motility [65].
Natural transformation: the uptake of extracellular DNA. This DNA can be bound and presumably transported to the periplasm by T4P. A distinct machinery transports this DNA to the bacterial cytoplasm where it can then be recombined with the host's DNA [41].
Pili: long and thin surface appendages found on many bacteria. They play major roles in colonization by facilitating adhesion, motility, DNA exchange, and protein uptake and secretion [11].
Type II secretion systems (T2SS): a secretor of many proteins, including virulence factors. It was shown to consist of components highly similar to those of T4P, and their systems have similar general architecture, all suggestive of an evolutionary relationship [44].
Transduction: bacteriophages can encapsulate segments of host DNA, which in turn can be injected into a new host where it can be recombined with the new host's DNA, leading to horizontal gene transfer [64].
Type IV pili (T4P): extracellular filaments of 6–8 nanometers in width and several micrometers in length. The T4P pilus is composed of PilA subunits that are assembled in a helical manner. T4P are involved in adherence, biofilm formation, motility, DNA uptake, and protein secretion.

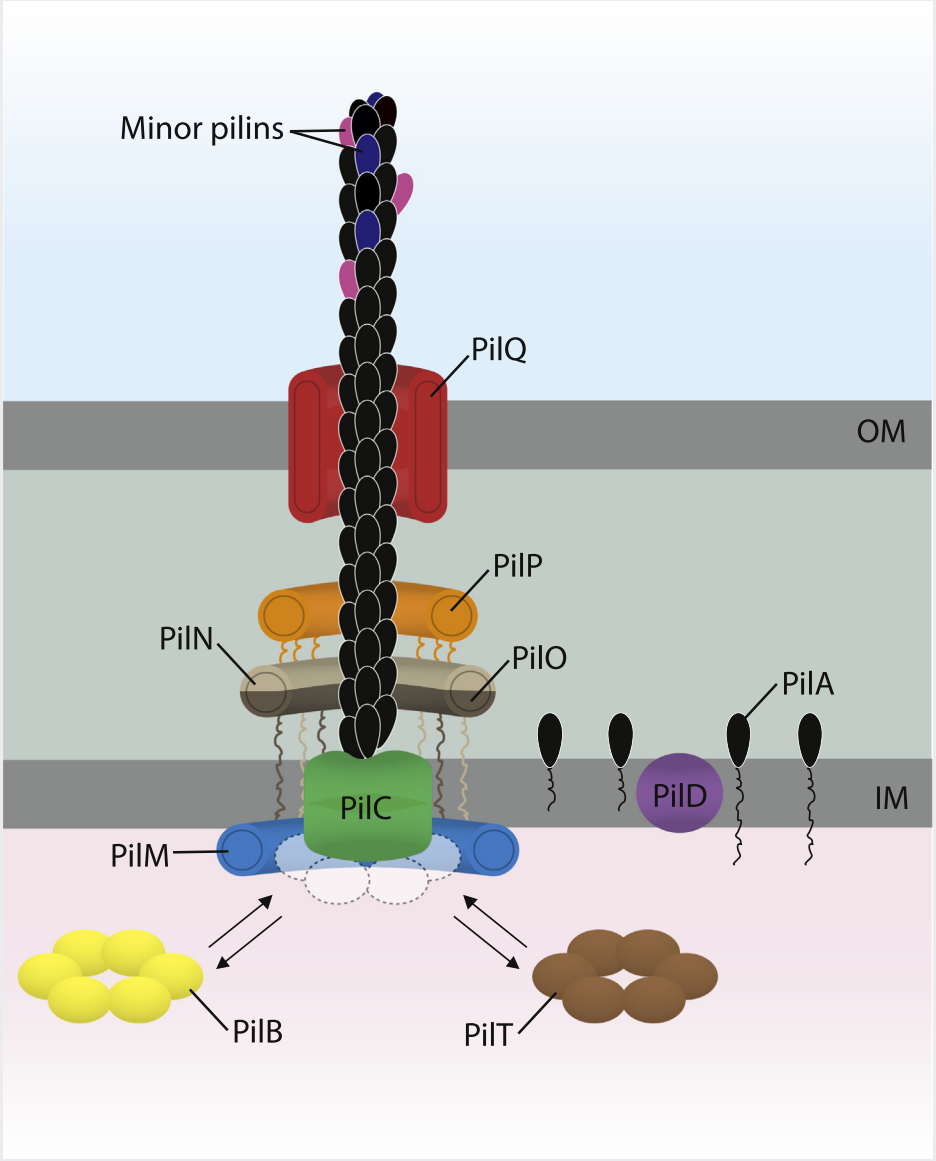

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Figure I. Type IV Pili Biogenesis in Gram-Negative Bacteria. After removal of the leader peptide by the prepilin peptidase PilD (purple), the major pilin subunits PilA (black) are incorporated into the growing pilus by platform protein PilC (green). The growing pilus is held in place by the PilMNOP alignment complex and is lead through the outer membrane pore formed by PilQ (red). Assembly of the pilin is driven by the cytoplasmic ATPase PilB (yellow), whilst disassembly or retraction is driven by the PilT ATPase (brown). Both ATPases can reversibly associate with the PilC protein. Finally, the minor pilins are thought to form a priming complex for pilin formation and can both end up at the pilus tip and along the pilus. Abbreviations: IM, inner membrane; OM, outer membrane. Figure references: [6,58,59].

Type IVa pili (T4aP): a very homogeneous and widespread subtype of type IV pili. Their major pilins are characterized by a shorter leader sequence (five or six amino acids) and shorter mature major pilin (~150 amino acids). Their biogenesis machinery typically harbors a PilT retraction ATPase [11].

Type IVb pili (T4bP): the major pilins of these pili have an amino acid leader sequence of 15–30 amino acids, and the mature major pilin ranges up to ~190 amino acids in length [11]. Until now, T4bP have been described only in diderms (Gram-negative bacteria) [3].

Type IVc pili (T4cP): tight-adherence (Tad) pili, previously described as a subtype of T4bP. T4cP are widespread, characterized by small major pilins, and their structural and biogenesis genes are encoded at a single locus [3].

[2,21]. The study of secreted and surface molecules of microbiota members is of special interest as these play key roles in the establishment of species in the versatile environment of the gut. The presence and importance of T4P in gut-relevant pathogens is substantiated by dedicated studies of some key species, such as pathogenic *E. coli* and *Clostridium* spp. However, no information exists on the

Key Figure

The Various Functions of Type IV Pili (T4P) in Gut-Related Species.

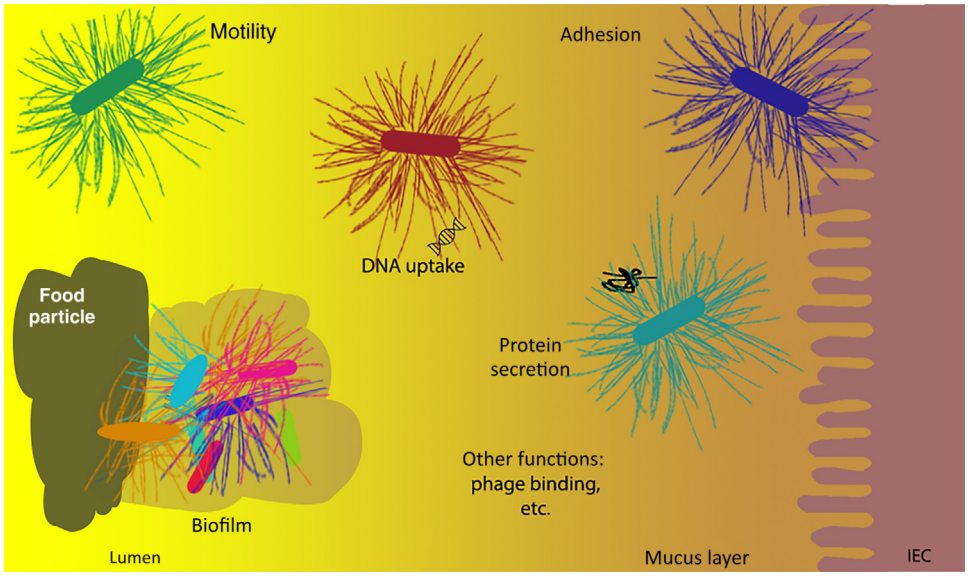

Figure 1. T4P-carrying cells are indicated by a cartoon of a bacillus covered in T4P throughout; this, however, is only one of the potential manifestations of T4P – which can also be less abundant and may be polarly localized. T4P have established roles in microbiota–host interactions, in both commensal and pathogenic bacteria. One of these roles is motility: bacteria use T4P to move around by growing their pilus, attaching it to a surface, and finally pulling themselves forward by retraction of the pilus. The T4P major pilin PilA, sometimes with the help of minor (tip) pilins, is also essential for adherence to surfaces and other bacteria, which can ultimately lead to biofilm formation. T4P also play a role in the exchange of molecules between the bacterium and its environment: T4P can bind and subsequently take up double stranded (ds)DNA and secrete proteins. In the latter process, growth of the pilus, through the membrane, pushes out proteins in the manner of a piston. It will be exciting to see if dedicated T4P functional studies uncover further roles for T4P in general, and in the specific context of the gut. Abbreviation: IEC, intestinal epithelial cell.

presence of T4P in gut commensals and, by extension, in the general context of a healthy gut microbiome. Starting from an earlier published set of 675 genomes of gut microbiome species [22], a total of 285 bacterial species were selected for which a complete genome was available (one strain per species). The probability that these species produce T4P was scored by evaluating the presence of genes encoding prepilins using the PilFind program [23] (1 if potential pilin-encoding genes were found, 0 when the program returned none) and IMG/MER (https://img.jgi.doe.gov) searches to find genes encoding conserved domains (Interpro) of biogenesis T4P proteins, exploiting their highly conserved domains (Box 2, and see Table S1 in the supplemental information online) [11]. A general cutoff at a score of 5 was used to identify a species as a T4P-producing candidate, as not all T4P biogenesis systems harbor PilT and given the low conservation of some biogenesis proteins such as PilN and PilP. This mining effort revealed that approximately 30% (86/285) of all tested microbial species harbor at least one major pilin candidate and at least four conserved biogenesis proteins. This estimation is likely conservative, as when a score cutoff of 4 is used for monoderms (mainly Gram-positive bacteria), which do not harbor the outer-membrane-located PilQ, this results in 132 species that likely produce T4P, augmenting the estimation to approximately 45% of bacteria potentially producing T4P in this microbiome dataset. The best-studied T4P-producing pathogens, such as *V. cholerae* and *P. aeruginosa* that also are found in

the gut, score 10 out of 10 in our analysis. From the members of six major intestinal phyla tested, the Proteobacteria harbor most T4P-encoding bacteria (37/69), with the most high-scoring species belonging to the genera *Acinetobacter*, *Campylobacter*, *Escherichia*, *Neisseria*, *Pseudomonas*, *Ralstonia*, *Vibrio*, and *Yersinia*. The phylum Fusobacteria also harbors many species predicted to produce T4P (5/7) as they harbor several conserved domains of biogenesis genes (Table S1). In addition, the phylum Firmicutes contains a significant number of 39 out of 144 species producing T4P, even 81/144 when considering the absence of outer-membrane proteins (i.e., a cutoff of 4). The phylum Actinobacteria generally scores low in this analysis, similarly to the phylum Bacteroidetes, where T4P-producing species seem to be less abundant. Further of note are the positive T4P scores of some important butyrate-producing gut bacteria, such as *Anaerostipes caccae* and *Eubacterium rectale,* as well as the Verrucomicrobia representative *Akkermansia muciniphila* [24]. It is to be expected that the scoring effort presented here can be further refined as recently suggested [3] and also applied to gut metagenomes, notably when these are generated by single-molecule sequencing approaches that preserve genomic continuity, since T4P genes are often scattered in the genome.

Functional Aspects of T4P in the Gut

Most knowledge on the functionality of T4P stems from analyses in pathogenic model organisms such as *P. aeruginosa, N. gonorrhoeae*, and *V. cholerae.* Given their ubiquity in gut, microbiota members' T4P are likely to be of general importance to microbiota–host interactions. However, original studies corroborating their role in beneficial gut microbiota members are scarce, possibly reflecting the limited attention for in-depth molecular and physiological analysis of individual gut anaerobes and the strong focus on omics studies in microbiome studies. Such analyses are highly interesting since T4P may have many gut-relevant functions, such as adhesion, motility, biofilm formation, and trafficking of molecules as discussed below (Figure 1).

Adherence

Adherence is the establishment of an interaction between microbes and their environment and one of the best studied features of T4P. Its importance in the context of the gut can hardly be overestimated: T4P can be involved in attachment to other cells (both bacterial and eukaryotic) and to food and fiber in the gut. The latter has been shown for the cellulose-binding T4P of *Ruminococcus albus,* a fiber-degrading species in the rumen of herbivores [25].

T4P-mediated adhesion can be driven by several proteins, such as the major pilins in *P. aeruginosa* [26] and the minor pilin PilV in *Neisseria meningitidis* which is essential for adherence to human endothelial cells [27]. Even nonpilin proteins can play a role, such as the secreted protein CofJ in ETEC, which bridges ETEC T4bP and the human host cell membrane [28]. Furthermore, ETEC T4P adhere stronger to intestinal cells (e.g., HT-29) than to other nonintestinal cell lines such as HeLa and Hep-2 (30–35% increase in adhesion) [29]. This indicates that T4P-mediated adhesion confers specificity and can aid in niche selection of bacteria in the context of the microbiota.

As substantiated in pathogens, attachment of bacteria to host cells via their T4P sets off a signaling cascade influencing gene expression, for example,. virulence-associated genes [30]. In the case of *P. aeruginosa*, a lung pathogen also residing in the human intestinal tract, T4aP-mediated adhesion to host cells results in a physiological response in the bacterium itself: tension, built up in the pili upon adhesion, induces conformational changes in the T4P machinery (Box 2) [31], which set off the production of two signaling molecules, cyclic adenosine monophosphate (cAMP) and cyclic diguanylate (c-di-GMP) [30]. Apart from modulating bacterial physiology, adhesion of bacteria via their T4P can result in signaling cascades conferring beneficial effects to the host. A potential example is reported in *Bifidobacterium breve*, where a Tad-pilus-associated protein, TadE, promotes

proliferation of colonic epithelial cell lines [32]. As *B. breve* is a colonizer of the infant gut, stimulation of colonic cell proliferation could enhance intestinal barrier maturation [32].

Biofilm Formation

Adhesion of bacteria can, in some cases, lead to **biofilm** formation, which is an agglomerate of numerous bacteria protected by a thick extracellular matrix. In the lumen of the gut, microcolony and biofilm formation can help to increase the residence time of both pathogenic and commensal bacteria, as well as promote nutrient exchange, horizontal gene transfer (linked to spread of antibiotic resistance), increase in survival rate, protection from physical stressors, etc. [1]. Biofilms also could have a role in pathogenic processes in the gut, as recently reviewed [1].

The role of T4bP in intestinal colonization and biofilm formation of *V. cholerae* [33] has been studied in depth (Figure 1). Amino acid residue substitutions at the C-terminal part of TcpA resulted in defective autoaggregation and microcolony formation by *V. cholerae,* and ultimately in a colonization defect in an infant mouse cholera model [33]. In *Clostridium difficile*, T4bP nonpiliated *pilA* mutants were hampered in adhesion and showed reduced biofilm formation on glass slides; this lead to biofilms with less biomass and fewer live cells [34]. Transcriptional upregulation by c-di-GMP of several T4P-related genes (*pilA* major pilin and *pilB* ATPase) promoted cell aggregation in *C. difficile* [35].

Twitching Motility

Motility is an advantageous feature for bacteria when colonizing a novel niche as it allows them to follow environmental cues and find novel interaction partners. Bacteria can move in a variety of ways, either passive (cell growth) or active (e.g., gliding, swarming) [36]. But T4P can also power motility (Figure 1), that is, twitching motility [36], as reported in gut pathogens such as *C. difficile* [37] and *N. gonorrhoeae* [38]. It is suggested that twitching motility is important for surface colonization; it increases the colonization area and thus might play a key role in the context of the gut microbiota [39].

DNA Uptake

DNA exchange can be beneficial for survival of (gut) bacteria as it can promote genetic diversity, for example, exchange of antibiotic-resistance cassettes [40]. DNA exchange between bacteria can occur through **conjugation**, **transduction**, and **natural transformation**. In the latter process, bacteria bind to DNA in the environment. The process is mediated by T4P and related filamentous structures (Figure 1), as observed in about 80 different species – notably studied in *Bacillus subtilis*, *Neisseria*, and *V. cholerae* [41]. Further investigation is necessary to see if, and how, members of the microbiota use T4P-mediated competence to exchange genetic material crucial for their colonization and impact on the host.

Protein Secretion

Bacteria secrete many functionally important molecules such as adhesins, proteases, toxins, and short-chain fatty acids [42], which are key to the establishment of microbial interactions in the gut. Proteins are often secreted through specific secretion systems such as **type II secretion systems (T2SS)**, Sec, or Tat systems [43]. T4P are evolutionarily related to T2SS and they share a similar general architecture and functionality as the T4P machinery can also secrete proteins [44]. In *V. cholerae*, T4bP are essential for the secretion of TcpF, a protein required for colonization of the infant mouse intestine [45]. This was illustrated by mutational analysis targeting T2SS, which still resulted in wild-type levels of TcpF in the culture supernatant [45]. Further mutations, abolishing the T4P machinery, confirmed the essentiality of T4P to TcpF secretion. Secretion is thought to take place by the help of a piston-like pilus that pushes the proteins through the PilQ outer membrane pore [46]. T4P protein secretion is not exclusive to

V. cholerae: it has also been reported in other bacteria such as animal gut pathogens *Dichelobacter nodosus* [47] and *Francisella tularensis* [48].

Concluding Remarks

T4P are key filaments that allow bacteria to interact with their environment, from abiotic surfaces to other microbes and host cells. Apart from adhesion, bacteria use their T4P to perform a range of different functions, from motility to molecule exchange (Figure 1). Many of these functionalities have been reported in gut pathogens, whilst reports in beneficial members of the gut microbiome are scarce, possibly since many of them are strict anaerobes. It is, however, likely that both commensals and pathogens residing in the gut exploit these functional features of T4P to a similar extent. Mining of 285 gut microbial species revealed that approximately 30%, and maybe even up to 45%, of the tested gut microbiota members harbor the potential to produce T4P. These numbers need to be further validated and substantiated as soon as more complete genomes are determined of key gut microbiome members (see Outstanding Questions). Furthermore, it is essential to establish whether the T4P genes are expressed and under which conditions. Once confirmed, functional studies can be undertaken to explore how T4P are used by beneficial microbiota members to establish themselves in the versatile context of the human gut. It is highly likely that a key role of T4P is in adhesion, niche selection, and establishment. Indeed, as shown for other types of pili on beneficial microbiota members, pili can play a role in competitive exclusion of pathogens from niches at the microbiota–host interface [49]. It will be interesting to explore whether the study of T4P, in the context of a healthy microbiota, reveals novel T4P functionalities and regulatory mechanisms – especially as some reports indicate that the oxygen concentration can influence T4P levels and functionality. In *N. gonorrhoeae*, anaerobic conditions severely inhibit twitching motility [50] and microcolony formation [51]. It remains to be further elucidated if this is a feature of pathogenic species and what could be the evolutionary basis for this intriguing finding.

In conclusion, we can state that T4P, and by extension pili in general, are key interaction filaments of bacteria, serving a wide range of functionalities. In the high-density microbial communities that reside in the gut, these filaments are crucial, and further research is necessary to fully understand their ubiquity, function, and importance in microbiota–host interactions. This will not only lead to a more detailed fundamental understanding of how bacteria influence host physiology but will also inevitably lead to novel therapeutic strategies to modulate microbiota composition and host health. Exciting times are ahead not only for our basic understanding of T4P functionality but also in identifying their role in microbiota–host interactions.

Outstanding Questions

How truly widespread are T4P, and pili in general, in the microbiomes of distinct niches of the human body? Can this be accurately estimated once single-molecule sequencing of metagenomes is within reach? And what does this teach us about the abundance of T4P and pili in general in commensals versus pathogens?

How can T4P of beneficial species of the microbiome affect host physiology? How do beneficial bacteria use their T4P to reach out to their environment and to modulate other microbes and host cells?

What are the signaling cascades affected by T4P, both in the bacterium carrying the T4P and affected organisms (microbial or mammalian origin)? What genes and molecules are typically involved in these processes? Can novel insights in these signaling cascades and molecules offer new therapeutic leads and targets? Can fundamental knowledge on T4P-mediated signaling be used for targeted microbiome engineering and host physiology modulation?

Given the similarity in biosynthesis and structure of T4P in general, how similar are T4P of commensal and pathogenic strains? Can this similarity be exploited in modulation of microbiome composition?

What other, yet to be discovered, functional aspects are carried out by T4P in the context of the gut beyond those discussed here?

How did evolution select for the diverse functions related to T4P? How are conserved T4P evolved to serve different purposes in different bacterial species?

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Supplemental Information

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