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The *Xanthomonas* type IV pilus

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Type IV pili, a special class of bacterial surface filaments, are key behavioral mediators for many important human pathogens. However, we know very little about the role of these structures in the lifestyles of plant-associated bacteria. Over the past few years, several groups studying the extensive genus of *Xanthomonas* spp. have gained insights into the roles of played by type IV pili in bacteria–host interactions and pathogenesis, motility, biofilm formation, and interactions with bacteriophages. Protein-protein interaction studies have identified T4P regulators and these, along with structural studies, have begun to reveal some of the possible molecular mechanisms that may control the extension/retraction cycles of these dynamic filaments.

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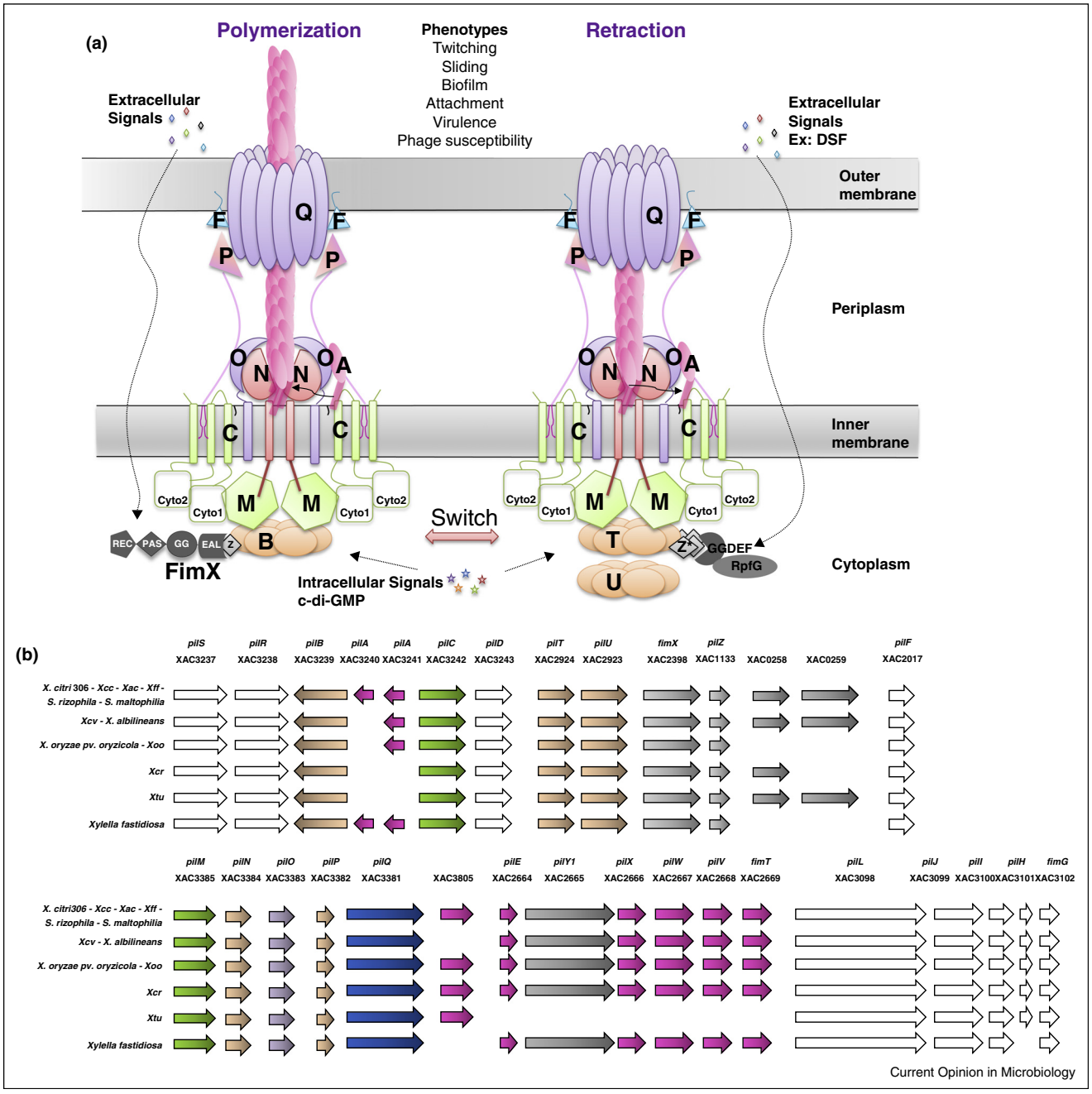
Introduction

Bacteria employ a wide range of strategies in order to survive in complex and ever-changing environments, both within and outside their hosts. This includes the production of extracellular hydrolytic enzymes and polysaccharides, adhesins and protein secretion systems that deliver virulence factors. Depending on conditions, cells have to oscillate between behaviors that involve attachment to surfaces (other bacteria, animal or plant tissue, inanimate matter) and moving across surfaces or through the liquid media, either as individual cells or in groups. One structure particularly important for these individual and group behaviors is the bacterial type IV pilus (T4P), a flexible surface filament 4–7 nm in diameter and several micrometers in length that can extend, attach to surfaces and retract, in this way facilitating bacterial movement, adhesion, orientation and multicellular organization. The function, structure and regulation of T4P have been extensively studied in genera such as *Pseudomonas*, *Neisseria*, *Escherichia* and *Vibrio* where they are important determinants of pathogenesis in human diseases, as well as in the social bacteria *Myxococcus xanthus* [1–8]. T4P have been less studied in plant pathogens, with the main exceptions being *Xylella* [9,10] and *Xanthomonas* species, both members of the Xanthomonadaceae family. Yellow-pigmented γ-proteobacteria of the genus *Xanthomonas* cause diseases in approximately 400 plant species. Pioneering works over the past 20 years have addressed some aspects of pilin gene expression and T4P production in *Xanthomonas* spp. [11–14]. The purpose of this review is to focus on recent studies that have revealed links between T4P function and *Xanthomonas* physiology and virulence as well as insights obtained from biochemical and structural studies into the molecular mechanisms of T4P regulation. Finally, we hope that this review will point to several key questions that urgently need to be addressed in order to have a more complete understanding of the regulation of this complex molecular machine.

Basic structural features of type IV pili

T4P are molecular nanomachines (Figure 1a) related to the ubiquitous type II secretion systems (T2SS) that translocate folded proteins from the periplasm across the cellular envelope of Gram-negative bacteria, as well as archaeal flagella [3,15,16]. In the case of T4P, the translocation substrates are principally pilin (PilA) subunits that form an extracellular helical polymer. Initially, prepilin subunits are translocated across the cytoplasmic (inner) membrane by the Sec system, their signal peptides are removed, subsequently methylated at the N-terminal Phe residue by the pre-pilin peptidase (PilD) [17] and the mature pilins are incorporated into the base of the growing pilus. We know very little about this process of pilus incorporation (and removal) except that it requires a so-called 'inner membrane platform' formed by integral proteins PilC, PilN, PilO, PilP and the cytoplasmic protein PilM [18] (Figure 1a). This platform interacts with the pilins and with the two specialized hexameric ATPases on the cytosolic face of the inner membrane: PilB and PilT [19,20]. PilB uses the energy of ATP hydrolysis to catalyze the incorporation of pilin subunits during pilus polymerization/extension while PilT catalyzes the removal of pilus subunits during depolymerization/retraction. One platform component,

Figure 1



The *Xanthomonas* type IV pilus. **(a)** T4P organization and regulation in *Xanthomonas* species. The T4P secretion machinery is made up of four subcomplexes: (i) the outer membrane subcomplex formed by the dodecameric ring of PilQ and the pilotin PilF, (ii) the inner membrane platform, made up of PilC, PilM, PilN, PilO and PilP, (iii) the ATPases PilB, PilT and PilU, and (iv) the pilus filament, a polymer of the major pilin, PilA, and minor pilins. In *Xanthomonas* species, T4P pilus biogenesis and function is thought to be regulated in part through a set of protein–protein interactions between the ATPases PilB, PilT and PilU and proteins containing c-di-GMP signaling domains at the base of the pilus. This hypothesis is based on interactions between FimX–PilZ–PilB observed in *X. citri* 306, FimX–PilZ interactions observed in *X. campestris* pv. *campestris* 17 and *X. oryzae* pv. *oryzae*, and interactions in *X. campestris* pv. *campestris* strain 8004 involving the c-di-GMP specific phosphodiesterase RpfG, two GGDEF proteins (XC_0249 and XC_0420), a PilZ domain protein (XC_2249) and PilT and PilU. These interaction networks could transmit intracellular signals (i.e. c-di-GMP) and extracelular signals (i.e. nutritional components, quorum sensing molecules such as DSF, or mechanical signals) to the regulatory ATPases. Pil proteins are represented by their distinguishing letter labels. Z and Z* are the PilZ domain proteins that interact with PilB or with PilT/PilU, respectively (see main text). **(b)** Organization of T4P-related genes in the Xanthomonadaceae family. Genes coding for T4P structural components and putative regulators are shown in a manner that reflects their organization in operons or gene clusters in different loci of the bacterial genomes. Specific gene clusters can be intuitively surmised from the gene numbers of *X. citri* 306 sequences indicated in the top row. Each row presents the common gene organization pattern observed for a set of representative members of the

Table 1

Specific conditions correlated with modified type IV pilus-related gene expression in *Xanthomonas* species

Mutant gene or growth conditions	*Xanthomonas* species and strain	Up-regulated or down-regulated T4P-related genes	Refs.
pilR	*X. axonopodis* pv. *citri* XW47	Down-regulated: *pilA*	[14]
rpfF	*X. citri* 306	Down-regulated: $pilA_{XAC3240}$ in exponential growth phase	[45•]
	X. campestris pv. *campestris XC1*	Down-regulated: *pilT* in middle exponential to early stationary phase	[44]
	X. hortorum pv. *pelargonii*	Down-regulated: *pilC* and *pilT* in early stage of infection	[46•]
rpfC	*X. hortorum* pv. *pelargonii*	Down-regulated: *pilC* and *pilT* in early stage of infection	[46•]
rpfG	*X. citri* 306	Up-regulated: $pilM_{XAC3385}$ in exponential growth phase	[45•]
In planta vs liquid culture	*X. citri* 306	Up-regulated: $pilA_{XAC3241}$	[26••]
		Down-regulated: $pilA_{XAC3240}$, *xac3805*[a]	
		Up-regulated: $pilD_{XAC3243}$, $pilM_{XAC3385}$ and $pilE_{XAC2664}$	[70]
Epiphytic vs liquid culture	*X. citri* 306	Up-regulated: $pilA_{XAC3241}$	[26••]
		Little or no change: $pilA_{XAC3240}$, *xac3805*[a]	

[a] The genes coding for XC_3823 and XAC3805 were originally annotated as *pilA*. However, major pilin *pilA* genes are normally found in a locus that also contains the *pilS*, *pilR*, *pilB*, *pilC* and *pilD* genes (Figure 1b), which is not the case for XC_3823 and XAC3805. The annotated GTG start codons of these genes are preceded by 106 in-frame codons that are well conserved in XC_3823/XAC3805 homologs in Xanthomonadaceae species. The N-terminal half of this extension (approximately 50 amino acids) corresponds to Pfam DUF4339, a domain of unknown function conserved in bacteria, archaea, and eukaryotes. Therefore, XC_3823, XAC3805 and their homologs are not PilA orthologs, but rather should be viewed as paralogs that may exert regulatory effects, possibly as minor pilins [26••].

PilP, a periplasmic inner membrane lipoprotein [21] interacts with PilQ, the T4P secretin, a dodecameric ring that creates a passage for the growing pilus to pass through the bacterial outer membrane [22•] (Figure 1a). In addition to the major pilin, minor pilins or pseudopilins participate in the priming of pilus assembly and may also be incorporated into the pilus fibre to modulate characteristics such as adhesion specificity, pilus length and the balance between pilus extension and retraction [23,24].

Type IV pilus genes and type IV pilus detection in *Xanthomonas* species

Most *Xanthomonas* spp. genomes sequenced to date code for homologs of all of the core structural components and transcription regulators of T4P biogenesis and function. As in other bacteria, these genes are arranged in clusters scattered throughout the bacterial genome (Figure 1b). Most *Xanthomonas* genomes code for two major pilin homologs coded in tandem in a cluster that also contains the *pilS*, *pilR*, *pilB*, *pilC* and *pilD* genes. Xanthomonadaceae family genomes also carry an operon that codes for homologs of the minor pilins PilE, PilX, PilW, PilV, FimT and the anti-retraction factor/adhesin PilY1 (Figure 1b). While T4P have been observed by electron microscopy *in Xylella fastidiosa* as thick, long, polar filaments [10,25], it is surprising that the only two reports of direct observation (via immunoelectron microscopy), of T4P in *Xanthomonas* species were carried out over 15 years ago in *X. campestris* pv. *hyacinthi* [12] and *X. campestris* pv. *vesicatoria* [11]. Detection of extracellular pilin subunits was subsequently reported in *X. citri* subsp. citri strain 306 (from here on denominated as *X. citri* 306) [26••].

The transcriptional regulation of T4P genes in *Xanthomonas* remains to be investigated in detail. Several large-scale transcriptomics studies have noted modifications in the expression levels of T4P-related genes in specific *Xanthomonas* mutants or in wild-type *Xanthomonas* cells under specific growth conditions (summarized in Table 1).

In *Pseudomonas aeruginosa*, the two-component system made up of the histidine kinase sensor protein PilS and the response regulator PilR controls transcription of the major pilin gene *pilA* [27]. In *X. axonopodis* pv. *citri* strain XW47, deletion of *pilR* eliminates *pilA* expression, while deletion of *pilS* did not significantly affect *pilA* expression [14]. In *X. campestris* pv. *vesicatoria* and *X. citri* 306, a palindromic sequence may form a hairpin transcriptional terminator in the intergenic region between the two pilin genes in the *pilSRBACD* locus (Figure 1b) and in both cases the upstream gene (*fimA* in *X.campestris* pv. *vesicatoria* [11] and $pilA_{XAC3241}$ in *X. citri* 306 [26••]) was observed to be expressed at higher levels than its adjacent downstream homolog. In the case of *X. citri* 306, $pilA_{XAC3241}$ expression is stimulated upon contact with citrus leaves, both epiphytically and *in planta* [26••].

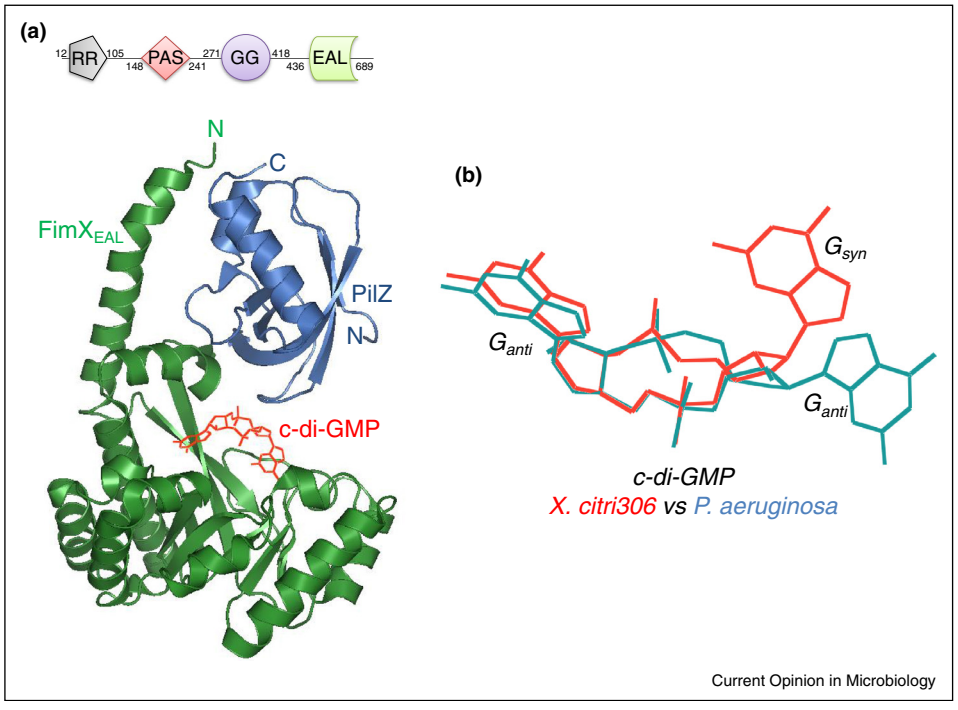
(Figure 1 Legend Continued) Xanthomonadaceae family of bacteria. *X. citri* subsp. *citri* strain 306 (*X. citri* 306), *X. campestris* pv. *campestris* strains 17, 8004 and ATCC33913 (*Xcc*), *Stenotrophomonas rizophila* strain DSM14405 (*S. rizophila*), *Stenotrophomonas maltophilia* strain JV3 (*S. maltophilia*), *X. axonopodis* pv. *citrumelo* strain F1 (*Xac*), *X. fuscans* subsp. *fuscans* strain 4837-R (*Xff*), *X. campestris* pv. *vesicatoria* strain 85-10 (*Xcv*), *X. albilineans* strain GPE PC73 (*X. albilineans*), *X. oryzae* pv. *oryzicola* strain CFBP2286 (*X. oryzae* pv. *oryzicola*), *X. oryzae* pv. *oryzae* strain PX086 (*Xoo*), *X. campestris* pv. *raphani* strain 756C (*Xcr*), *X. translucens* pv. *undulosa* strain Xtu 4699 (*Xtu*) and *Xylella fastidiosa* strain 9a5c (*Xylella fastidiosa*).

Post-transcriptional regulation of T4P function by c-di-GMP metabolizing proteins and receptors

The pilus polymerization and depolymerization process is regulated in part by cytoplasmic hexameric proteins belonging to the AAA+ (ATPase associated with cellular activities) family [28]. PilB, a homolog of the T2SS ATPase (XpsE in *X. campestris* pv. *campestris*), is required for pilus polymerization, while PilT is required for pilus retraction [19,20]. Interestingly, PilT lacks an N-terminal domain present in PilB and XpsE [29]. PilU, a PilT paralog, has also been implicated in T4P function in *P. aeruginosa*, although its precise role is unclear [8,20,30]. Each of the principle model organisms for which T4P have been studied in detail — *P. aeruginosa*, *Neisseiria* spp., *Synechocystis*, *Vibrio cholerae* and *Myxococcus xanthus* — present regulatory mechanisms with surprisingly unique aspects (reviewed in [7,8] and see comments below). Although we are still only beginning to get a glimpse of these mechanisms in *Xanthomonas* spp., the evidence so far suggests that they will also be a source of novelty.

PilZ and FimX are two regulators of T4P biogenesis in *Xanthomonas* species and are homologs of the T4P biogenesis regulators of the same name initially characterized in *P. aeruginosa* [31,32]. PilZ belongs to a large family of proteins that are receptors for *bis* (3 → 5) cyclic dimeric guanosine monophosphate (c-di-GMP), though the PilZ protein itself does not bind this second messenger [33,34]. FimX is a large protein with REC, PAS, GGDEF, and EAL domains (Figure 2). The N-terminal REC domain of FimX lacks a conserved aspartic acid residue required for phosphorylation by cognate histidine kinases. The PAS domains are commonly found in signaling proteins where they sense a variety of different stimuli including oxygen, redox potential and light [35], though its specific ligand in FimX, if any, is unknown. The GGDEF and EAL domains of FimX are inactive diguanylate cyclase and c-di-GMP phosphodiesterase domains, but the FimX EAL domain has retained the ability to bind c-di-GMP [36••,37••,38••]. Interestingly c-di-GMP binds to both *X. citri* 306 and *X. campestris* FimX in a conformation in which one of the N-glycosidic bonds is in a *syn* conformation [36••,38••] contrary to the all-*anti* conformation observed for the ligand when bound to *P. aeruginosa* FimX (Figure 2). To date, these are the only examples of *syn* c-di-GMP conformers in protein-c-di-GMP complexes. Furthermore, though no *in vitro* interactions have been observed between the

Figure 2



PilZ–FimX$_{EAL}$–c-di-GMP complex structure and the rare conformation of the c-di-GMP ligand. **(a)** Crystal structure of the PilZ–FimX$_{EAL}$–c-di-GMP complex from *X. citri* 306 (PDBID: 4FOU) [36••]. PilZ (blue), the EAL domain of FimX (residues from 426 to 689, green) are shown in cartoon. The c-di-GMP ligand is shown in red. The domain architecture of the FimX protein is shown above: RR, REC or response regulator domain; PAS, PAS domain; GG, GGDEF domain; EAL, EAL domain. Amino acid residues that delimit the predicted domains are indicated (numbers correspond to the FimX protein (XAC2398) from *X. citri* 306). **(b)** Superposition of the c-di-GMP ligands observed in the *X. citri 306* FimX$_{EAL}$–c-di-GMP and PilZ–FimX$_{EAL}$–c-di-GMP complexes (red) with that observed in the *P. aeruginosa* FimX$_{EAL}$ structure (blue, PDBID: 3HV8). Both of the guanine bases in *P. aeruginosa* FimX are in the *anti* conformation (G_{anti}) while in *Xac* FimX the bound c-di-GMP molecule has one guanine base in the *anti* conformation and the other in the *syn* orientation (G_{syn}).

P. aeruginosa PilZ and FimX proteins [39], stable complexes were observed for the two proteins from *X. citri* 306 [33,36••,39], *X. campestris* pv. *campestris* 17 [38••] and *Xanthomonas oryzae* pv. *oryzae* [37••]. *X. citri* 306 PilZ interacts with the FimX EAL domain ($FimX_{EAL}$) in the presence and in the absence of c-di-GMP [36••,39] and the crystallographic structures of PilZ–$FimX_{EAL}$–c-di-GMP complexes from *X. citri* 306 (Figure 2a, [36••]) and *X. campestris* pv. *campestris* 17 [38••] have been solved. In addition to its interactions with FimX, *X. citri* 306 PilZ interacts with PilB and a stable FimX–PilZ–PilB ternary complex has been observed [33]. PilZ–PilB interactions are mediated in part through a PilZ region made up of 10 well-conserved C-terminal residues which are unstructured and therefore not visible in the PilZ and PilZ–$FimX_{EAL}$–c-di-GMP crystal structures [33,36••,38••]. FimX–PilZ–PilB interactions could be involved in the regulation of PilB function, where specific signals sensed by FimX domains could be transmitted via PilZ in *X. citri* 306 and related bacterial species (Figure 1a). Knockouts of these proteins, or mutations in PilZ residues necessary for interactions with FimX or PilB abolish T4P biogenesis, twitching motility, bacteriophage infection and plant adherence in *X. citri* 306 and *X. oryzae* pv. *oryzae* [26••,33,36••,37••,38••] and a knockout of Filp, the FimX ortholog in *X. oryzae* pv. *oryzae*, presents reduced virulence in host plants and reduced hypersensitive response in non-host plants [37••] (Table 2). The role of c-di-GMP in the control of the FimX–PilZ–PilB complex is not yet clear. While on the one hand mutations that interfere in c-di-GMP binding by FimX reduce affinity for PilZ [36••,38••,39], on the other hand Qi *et al.* [39] observed that there is no significant difference in the dissociation constants for the PilZ–FimX complex in both the presence and in the absence of c-di-GMP. Until now no FimX mutant that is unable to bind c-di-GMP but retains the ability to bind PilZ has been isolated and so a separation of the physiological effects of these two FimX properties has not yet been achieved.

Another set of studies in *X. campestris* pv. *campestris* 8004 identified a direct relationship between the diffusible signaling factor (DSF)-mediated quorum sensing pathway and the PilT and PilU ATPases [40••,41,42]. DSF synthesis by RpfF and binding to RpfC results in c-di-GMP hydrolysis by the RpfG phosphodiesterase and knockouts of the *rpfF*, *rpfC* and *rpfG* genes in *X. campestris* pv. *campestris* result in increased c-di-GMP levels (due to the loss in RpfG phosphodiesterase activity) and in cellular aggregation [41,43]. Furthermore, knockouts in the *rpfF* and *rpfC* genes in *X. campestris* pv. *campestris* XC1 [44], *X. citri* 306 [45•] and *X. hortorum* pv. *pelargonii* [46•] result in the down-regulation of the expression of some T4P-related genes (Table 1). (Similarly, increased c-di-GMP levels have been recently correlated with reduced *pilA* transcription and T4P biogenesis in *M. xanthus* [47].) Ryan *et al.* identified a dynamic set of protein–protein interactions that involve RpfG, two GGDEF proteins (XC_0249 and XC_0420), a PilZ domain protein (XC_2249) and PilT and PilU [40••,41,42]. Homologous interactions have been observed between *X. citri* 306 RpfG and the GGDEF domain-containing proteins XAC0258 and XAC0424 [48]. Ryan *et al.* went on to show that the DSF-dependent *in vivo* co-localization of the above-mentioned proteins and DSF-dependent regulation of motility are surprisingly not dependent on the diguanylate cyclase activities of XC_0249 and XC_0420 nor on c-di-GMP binding by XC_2249 [40••]. So, again, as we saw for the FimX–PilZ–PilB complex, the precise role of c-di-GMP in this DSF-mediated branch of the T4P regulatory pathway is also still not well delimited. Of course, c-di-GMP binding and diguanylate cyclase activity could be important under specific physiological conditions that have not yet been investigated.

The (still incomplete) picture that is emerging from these studies suggests that the *Xanthomonas* PilB, PilT and PilU ATPases are regulated by a complex set of protein–protein interactions modulated by c-di-GMP and/or other metabolic or environmental signals (Figure 1a) and that the precise role of c-di-GMP in this process may be too complex to be described simply as an 'activator' or 'inhibitor' of T4P biogenesis, T4P-mediated 'motility' or other functions. For example, as will be discussed further below, T4P are required for both the 'go-it-alone' motility of individual cells at the expanding fringe of twitching zones but are also essential for the coordinated motility required for the formation of complex multicellular 3D structures in mature biofilms (often regarded, perhaps erroneously, as 'sessile' behavior).

Finally, it is worthwhile to mention some recent studies that have demonstrated the direct participation of c-di-GMP in the regulation of T4P in other bacteria. An intriguing study has shown that c-di-GMP binds directly to the PilB homolog MshE, an ATPase associated with the mannose-sensitive hemagglutinin T4P of *Vibrio cholerae* [49••]. Interestingly, *Vibrio* spp. do not possess orthologs of the *X. citri* 306 (XAC1133) and *P. aeruginosa* (PA2960) PilZ proteins. Furthermore, *Pseudomonas* PilB does not bind c-di-GMP directly [49••] and residues required for c-di-GMP binding in MshE and its closest homologs are not conserved in PilB proteins in *Xanthomonas* and *Pseudomonas* species (data not shown). We can gather from these observations that c-di-GMP and/or c-di-GMP-binding proteins interface with T4P ATPases in *Xanthomonas* spp., *P. aeruginosa* and *Vibrio cholerae* through significantly different mechanisms, revealing a large amount of plasticity in the evolution of these regulatory pathways. Finally, another exciting study has shown that in the Gram-positive bacteria *Clostridium difficile*, the operon coding for a set of T4P genes, including the genes for the major pilin and the PilB ATPase, is preceded by a c-di-GMP riboswitch that acts as a transcription terminator at low c-di-GMP levels and that c-di-GMP binding

Table 2

Type IV Pilus-related phenotypes in observed in *Xanthomonas* species

T4P-related mutant gene	*Xanthomonas* species and strain	T4P-related phenotype						
		Twitching motility	Surface sliding/spreading motility	Biofilm	Attachment	Virulence/ pathogenesis	Phage susceptibility	Other phenotypes
pilA	*X. campestris* pv. *vesicatoria* 3240				[11]			[11][d]
	X. citri 306	[26••]	[26••]	[26••]	[26••]		[26••]	
	X. fuscans subsp. *fuscans* CFBP4834-R					[71]		[71][e]
	X. campestris pv. *citri* XW47						[13]	
	Xanthomonas EC-12	[62]					[62]	
XC_3823 minor pilin[a]	*X. campestris* pv. *campestris* 8004		[54,55]					
pilC	*X. campestris* pv. *campestris* 8004					[72]		
pilQ	*X. oryzae* pv. *oryzae* BXO43					[68]		[68][f]
	X. oryzae pv. *oryzae* KACC10331	[52]		[52]		[52]		
	X. oryzae pv. *oryzicola* BLS303					[67]		
pilB	*X. citri* 306	[26••]	[26••,33]	[26••,59]	[26••]		[26••]	
	X. campestris pv. *campestris* 8004					[72]		
pilZ[b]	*X. citri* 306	[26••]	[26••,33]	[26••]	[26••]		[26••]	
	X. oryzae pv. *oryzae* PX099		[34]			[34,37••]		[37••][g]
	X. oryzae pv. *oryzicola* BLS303					[67]		
	X. campestris pv. campestris 8004 (XC_3221)		[55]			[55]		
fimX	*X. citri* 306	[26••]	[26••,33]	[26••]	[26••]		[26••]	
	X. oryzae pv. *oryzae* PX099		[37••]			[37••]		[37••][g]
pilM	*X. oryzae* pv. *oryzicola* BLS303					[67]		
pilY1	*X. oryzae* pv. *oryzicola* BLS303					[67]		
pilT	*X. citri* 306	[26••]					[26••]	
	X. oryzae pv. *oryzicola* BLS303					[67]		
	X. campestris pv. *campestris* 8004		[40••]					
pilU	*X. campestris* pv. *campestris* 8004		[40••]					
XC_2249 (pilZ paralog)[c]	*X. campestris* pv. *campestris* 8004		[40••,55]			[55]		
XC_0249/XC_0420 (diguanylate cyclases)[h]	*X. campestris* pv. *campestris* 8004		[41]			[41]		
pilR	*X. axonopodis* pv. *citri* XW47						[14]	

[a] See footnote (a) of Table 1.
[b] Gene product interacts with FimX and PilB in *X. citri* 306 [33].
[c] Gene product interacts with PilT, PilU and the GGDEF-domain proteins XC_0249 and XC_0420 in *X. campestris* pv. *campestris* 8004 [40••].
[d] Aggregation.
[e] Transmission.
[f] Lesion length and migration.
[g] Reduced levels of specific *hrp* gene transcripts.
[h] XC_0249 and XC_0420 interact with RpfG and XC_2249 in *X. campestris* pv. *campestris* 8004. Moderate effects are observed for the single XC_0249 or XC_0420 mutants while more drastic effects are observed for the double mutant strain [41].

results in increased read-through and production of full-length transcripts [50].

T4P-dependent phenotypes in *Xanthomonas* species

Several specific T4P-dependent phenotypes that have been observed in *Xanthomonas* species are summarized in Table 2 and are discussed below.

Twitching and sliding motilities

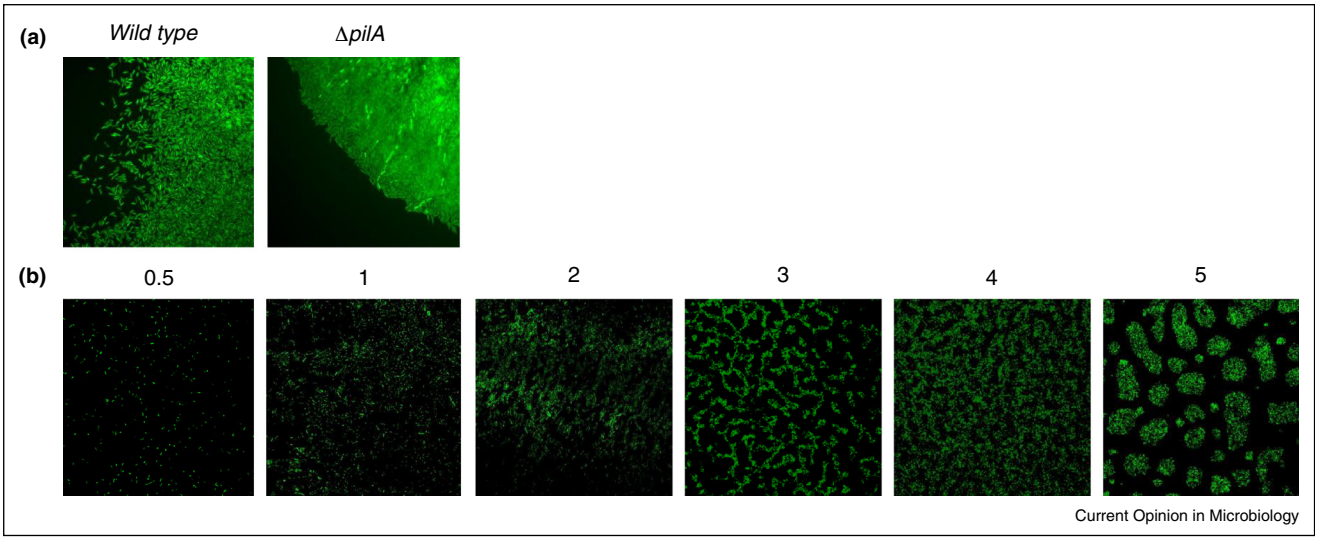
Twitching motility is a form of bacterial translocation over moist organic and inorganic surfaces [51] that is mediated by the extension, attachment, and subsequent retraction of T4P and has been well described in different bacteria such as *Pseudomonas*, *Myxococcus xanthus* (S-motility), *Neisseria gonorrhoeae* and *Xylella fastidiosa* [7–9,25]. Subsurface twitching (in which bacteria migrate on a solid surface interfaced with a layer of agar) has been described in *X. citri* 306 [26••] and *X. oryzae* pv. *oryzae* [52]. In such experiments, the edge of the expanding colony (the twitching zone) is made up of small groups or individual cells with a poorly defined and irregular boundary. On the other hand, T4P mutant *X. citri* 306 and *X. oryzae* pv. *oryzae* cells present a more uniform and well-defined boundary, with tightly packed cells [26••,52] (Figure 3a).

Another type of motility that has been observed to be influenced by T4P is sliding (or spreading) motility, a passive, T4P-independent and flagella-independent transport mechanism across moist surfaces, correlated with the secretion of surface-wetting substances, that harnesses the expansive forces of a growing colony [53]. The general observation is that *X. citri* 306 and *X. oryzae* pv. *oryzae* mutants lacking a functional T4P display significantly increased sliding on semi-solid agar surfaces [26••,33,34,37••]. On the other hand, T4P-deficient *X. campestris* pv. *campestris* mutants display reduced spreading [40••,41,54,55] (Table 2). These differences are difficult to explain at the moment, but the absence of a functional T4P on the bacterial surface will force upon the bacteria harsh lifestyle restrictions that could be expected to trigger complex metabolic and physiological responses (e.g., stress, surfactant secretion or the activation or deactivation of other surface structures) under specific conditions. These responses could vary significantly from species to species.

T4P-dependent biofilm formation and surface attachment

Bacterial biofilms are highly organized and topologically complex cellular populations composed of a matrix of bacteria, proteins, polysaccharides and extracellular DNA. Biofilm formation has been studied in several *Xanthomonas* species, including *X. oryzae* pv. *oryzae* [52], *X. campestris* pv. *campestris* [56], *X. axonopodis* pv. *phaseolus* var. fuscans [57] and *X. citri* 306 [26••,58–60] and in some cases *Xanthomonas* species with mutations in T4P-related

Figure 3



Xanthomonas citri subsp. *citri* subsurface twitching motility and biofilm development. **(a)** Microscopic images of the edge of the subsurface twitching zone of wild type and Δ*pilA X. citri* 306 strains expressing green fluorescent protein. *X. citri* 306 cells were stab inoculated through a thin layer of nutrient agar and incubated at 28 °C for 2 days. Bacteria were observed by confocal microscopy at 100× magnification. Images show the fringes of the twitching zones at the interstitial surfaces between the glass base of the microscopy chamber and nutrient-agar medium. Note that the edge of the twitching zone of wild-type *X. citri* 306 is made up of small groups or individual cells that have separated from the main body of the colony. In contrast, Δ*pilA* strain presents a more uniform and well-defined boundary with tightly packed cells [26••]. **(b)** Development of an *X. citri* 306 biofilm. *X. citri* 306 was inoculated in King's broth medium supplemented with 2% glucose and incubated in a microscopy chamber at 28 °C. Images of the *x*–*y* plane of the developing biofilm on the chamber surface were taken over a period of 5 days (post-inoculation time in days indicated above each image). The mature biofilm is characterized by well-separated three-dimensional, columnar or mushroom-like structures (seen from above in these images). These structures are largely absent in biofilms formed by T4P-deficient *X. citri* 306 strains [26••].

genes have presented reduced biofilm formation and surface adherence (Table 2). Figure 3b shows how *X. citri* 306 biofilm passes through several stages of maturation, eventually producing tri-dimensional and robust columnar mushroom-like structures in the mature biofilm [26••,58]. Malamud et al. showed that *Xanthomonas* biofilms are dynamic communities, with small groups of 'pioneer' cells splitting off to inhabit new environments [58]. T4P-deficient *X. citri* 306 strains are unable to form the typical mushroom-like structures of a mature biofilm [26••].

T4P-dependent bacteriophage infection

Bacteria–bacteriophage interactions are increasingly being explored as a potential means of combating the spread of phytopathogenic bacteria in crops. Several bacteriophages have been identified infecting members from the Xanthomonadaceae family, including *X. oryzae* pv. *oryzae* [61], *X. citri* [13,14,26••,62–65], *Xanthomonas* strain EC-12, *Xylella fastidiosa* [62] and *Stenotrophomonas maltophilia* [66]. A requirement of T4P has been observed for the infection of several *Xanthomonas citri* strains by a variety of bacteriophages [13,14,26••,62] and some phages have been observed to interfere with bacterial motility and the T4P. For example, infection with the phage XacF1 affects *X. citri* MAFF301080 twitching and T4P production [65].

T4P as virulence factors in Xanthomonas species

The role of T4P in bacteria–host interactions varies among *Xanthomonas* species. A T4P-deficient *fimA (pilA)* mutant strain of *X. campestris* pv. *vesicatoria* produced normal disease symptoms when inoculated into tomato plants [11]. Similarly, T4P-deficient *X. citri* 306 mutants seem unhindered in their ability to induce citrus canker symptoms as well as in survival both on and within the host plant [26••]. On the other hand, mutations in the genes *pilQ*, *pilZ*, *pilT*, *pilM* and *pilY1* from the rice plant pathogen *X. oryzae* pv. *oryzicola* resulted in modest to severe reductions in virulence when inoculated in host plants [67]. In *X. oryzae* pv. *oryzae*, bacterial attachment, entry, *in planta* migration and virulence in host rice plants was reduced in T4P-deficient *pilQ* mutants [52,68] and knockouts in the homologs of the T4P regulators FimX (Filp) and PilZ (PXO_02715) of this bacteria resulted in reduced virulence on rice and reduced hypersensitive response induction in non-host tobacco [37••] (Table 2).

Interestingly, Yang *et al.* [37••] reported that the Filp-deficient and PXO_02715-deficient strains presented reduced levels of specific *hrp* gene transcripts (*hrpX*, *hrpG and hpa1*), some of which that control the expression of the Type III secretion system, a major virulence factor in most *Xanthomonas* spp. pathogens. It is not clear at the moment whether Filp and PXO_02715 directly control *hrp* gene expression or whether this is part of a more general response to the changes in lifestyle forced upon the cell by the absence of a functional T4P. In fact, in *P. aeruginosa*, the attachment of T4P to a solid surface followed by pilus retraction triggers signal transduction through a mechanically-induced chemotaxis-like sensory system that regulates the transcription of hundreds of genes, including virulence factors [69].

Future perspectives

We conclude by posing a some open questions that should orient future studies: (1) What are the signal transduction networks that control T4P gene expression in *Xanthomonas* spp? (2) What are the post-transcriptional signal transduction networks that control T4P biogenesis, extension, length and retraction? (3) What is the role played by c-di-GMP as a regulator of transcription and/or protein–protein interactions in the above mentioned pathways? (4) What are the roles of the conserved minor pilins (PilE, PilV, PilW, PilX, FimT, and the second copy of PilA) in Xanthomonadaceae species? (5) What is the role of PilU, the third and poorly understood ATPase? (6) What is the interplay between PilB–PilZ–FimX interactions observed in *X. citri* 306 (and the homologous PilZ–FimX interactions observed in *X. campestris* pv. *campestris* and *X. oryzae* pv. *oryzae*) and the loosely analogous interactions involving PilT and GGDEF-domain and PilZ-domain-containing proteins observed in *X. campestris* pv. *campestris*? (7) How do specific environmental signals (nutritional conditions, cell density, and soluble and cell contact-dependent intercellular signals) control twitching motility and other T4P-dependent functions in *Xanthomonas* species? (8) How do T4P contribute to *Xanthomonas* competitiveness and survival in antagonistic interactions with other bacterial species and eukaryotic microbes?

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Mattick JS: **Type IV pili and twitching motility**. *Annu Rev Microbiol* 2002, **56**:289-314.
2. Burrows LL: ***Pseudomonas aeruginosa* twitching motility: type IV pili in action**. *Ann Rev Microbiol* 2012, **66**:493-520.
3. Craig L, Li J: **Type IV pili: paradoxes in form and function**. *Curr Opin Struct Biol* 2008, **18**:267-277.
4. Eriksson J, Eriksson OS, Maudsdotter L, Palm O, Engman J, Sarkissian T, Aro H, Wallin M, Jonsson AB: **Characterization of motility and piliation in pathogenic Neisseria**. *BMC Microbiol* 2015, **15**:92.
5. Seitz P, Blokesch M: **DNA-uptake machinery of naturally competent *Vibrio cholerae***. *Proc Natl Acad Sci U S A* 2013, **110**:17987-17992.
6. Friedrich C, Bulyha I, Sogaard-Andersen L: **Outside-in assembly pathway of the type IV pilus system in *Myxococcus xanthus***. *J Bacteriol* 2014, **196**:378-390.

7. Maier B, Wong GC: **How bacteria use type IV pili machinery on surfaces**. *Trends Microbiol* 2015, **23**:775-788.

8. Leighton TL, Buensuceso RN, Howell PL, Burrows LL: **Biogenesis of *Pseudomonas aeruginosa* type IV pili and regulation of their function**. *Environ Microbiol* 2015, **17**:4148-4163.

9. Li Y, Hao G, Galvani CD, Meng Y, De La Fuente L, Hoch HC, Burr TJ: **Type I and type IV pili of *Xylella fastidiosa* affect twitching motility, biofilm formation and cell–cell aggregation**. *Microbiology* 2007, **153**:719-726.

10. Meng Y, Li Y, Galvani CD, Hao G, Turner JN, Burr TJ, Hoch HC: **Upstream migration of *Xylella fastidiosa* via pilus-driven twitching motility**. *J Bacteriol* 2005, **187**:5560-5567.

11. Ojanen-Reuhs T, Kalkkinen N, Westerlund-Wikstrom B, van Doorn J, Haahtela K, Nurmiaho-Lassila EL, Wengelnik K, Bonas U, Korhonen TK: **Characterization of the fimA gene encoding bundle-forming fimbriae of the plant pathogen *Xanthomonas campestris* pv. *vesicatoria***. *J Bacteriol* 1997, **179**:1280-1290.

12. van Doorn J, Boonekamp PM, Oudega B: **Partial characterization of fimbriae of *Xanthomonas campestris* pv. *hyacinthi***. *Mol Plant-microbe Interact* 1994, **7**:334-344.

13. Su WC, Tung SY, Yang MK, Kuo TT: **The pilA gene of *Xanthomonas campestris* pv. *citri* is required for infection by the filamentous phage cf**. *Mol Gen Genet* 1999, **262**:22-26.

14. Yang YC, Chou CP, Kuo TT, Lin SH, Yang MK: **PilR enhances the sensitivity of *Xanthomonas axonopodis* pv. *citri* to the infection of filamentous bacteriophage Cf**. *Curr Microbiol* 2004, **48**:251-261.

15. Ghosh A, Albers SV: **Assembly and function of the archaeal flagellum**. *Biochem Soc Trans* 2011, **39**:64-69.

16. Peabody CR, Chung YJ, Yen MR, Vidal-Ingigliardi D, Pugsley AP, Saier MH Jr: **Type II protein secretion and its relationship to bacterial type IV pili and archaeal flagella**. *Microbiology* 2003, **149**:3051-3072.

17. Strom MS, Nunn DN, Lory S: **A single bifunctional enzyme, PilD, catalyzes cleavage and N-methylation of proteins belonging to the type IV pilin family**. *Proc Natl Acad Sci U S A* 1993, **90**:2404-2408.

18. Tammam S, Sampaleanu LM, Koo J, Manoharan K, Daubaras M, Burrows LL, Howell PL: **PilMNOPQ from the *Pseudomonas aeruginosa* type IV pilus system form a transenvelope protein interaction network that interacts with PilA**. *J Bacteriol* 2013, **195**:2126-2135.

19. Chiang P, Habash M, Burrows LL: **Disparate subcellular localization patterns of *Pseudomonas aeruginosa* Type IV pilus ATPases involved in twitching motility**. *J Bacteriol* 2005, **187**:829-839.

20. Chiang P, Sampaleanu LM, Ayers M, Pahuta M, Howell PL, Burrows LL: **Functional role of conserved residues in the characteristic secretion NTPase motifs of the *Pseudomonas aeruginosa* type IV pilus motor proteins PilB, PilT and PilU**. *Microbiology* 2008, **154**:114-126.

21. Drake SL, Sandstedt SA, Koomey M: **PilP, a pilus biogenesis lipoprotein in *Neisseria gonorrhoeae*, affects expression of PilQ as a high-molecular-mass multimer**. *Mol Microbiol* 1997, **23**:657-668.

22. • Gold VA, Salzer R, Averhoff B, Kuhlbrandt W: **Structure of a type IV pilus machinery in the open and closed state**. *Elife* 2015:4.
In situ structure of a T4P molecular machine, in the open and closed states, determined by electron cryo-tomography of whole *Thermus thermophilus* cells.

23. Nguyen Y, Sugiman-Marangos S, Harvey H, Bell SD, Charlton CL, Junop MS, Burrows LL: ***Pseudomonas aeruginosa* minor pilins prime type IVa pilus assembly and promote surface display of the PilY1 adhesin**. *J Biol Chem* 2015, **290**:601-611.

24. Wolfgang M, van Putten JP, Hayes SF, Koomey M: **The comP locus of *Neisseria gonorrhoeae* encodes a type IV prepilin that is dispensable for pilus biogenesis but essential for natural transformation**. *Mol Microbiol* 1999, **31**:1345-1357.

25. Cursino L, Galvani CD, Athinuwat D, Zaini PA, Li Y, De La Fuente L, Hoch HC, Burr TJ, Mowery P: **Identification of an operon, Pil-Chp, that controls twitching motility and virulence in *Xylella fastidiosa***. *Mol Plant Microbe Interact* 2011, **24**:1198-1206.

26. •• Dunger G, Guzzo CR, Andrade MO, Jones JB, Farah CS: **Xanthomonas citri subsp. citri type IV pilus is required for twitching motility, biofilm development, and adherence**. *Mol Plant Microbe Interact* 2014, **27**:1132-1147.
Characterization of mutations in *pilA*, *pilB*, *pilT*, *fimX* and *pilZ* on *X. citri* 306 subsurface twitching mobility, biofilm development and susceptibility to phage infection.

27. Hobbs M, Collie ES, Free PD, Livingston SP, Mattick JS: **PilS and PilR, a two-component transcriptional regulatory system controlling expression of type 4 fimbriae in *Pseudomonas aeruginosa***. *Mol Microbiol* 1993, **7**:669-682.

28. Pena A, Arechaga I: **Molecular motors in bacterial secretion**. *J Mol Microbiol Biotechnol* 2013, **23**:357-369.

29. Satyshur KA, Worzalla GA, Meyer LS, Heiniger EK, Aukema KG, Misic AM, Forest KT: **Crystal structures of the pilus retraction motor PilT suggest large domain movements and subunit cooperation drive motility**. *Structure* 2007, **15**:363-376.

30. Whitchurch CB, Mattick JS: **Characterization of a gene, pilU, required for twitching motility but not phage sensitivity in *Pseudomonas aeruginosa***. *Mol Microbiol* 1994, **13**:1079-1091.

31. Alm RA, Bodero AJ, Free PD, Mattick JS: **Identification of a novel gene, pilZ, essential for type 4 fimbrial biogenesis in *Pseudomonas aeruginosa***. *J Bacteriol* 1996, **178**:46-53.

32. Huang B, Whitchurch CB, Mattick JS: **FimX, a multidomain protein connecting environmental signals to twitching motility in *Pseudomonas aeruginosa***. *J Bacteriol* 2003, **185**:7068-7076.

33. Guzzo CR, Salinas RK, Andrade MO, Farah CS: **PILZ protein structure and interactions with PILB and the FIMX EAL domain: implications for control of type IV pilus biogenesis**. *J Mol Biol* 2009, **393**:848-866.

34. Yang F, Tian F, Chen H, Hutchins W, Yang CH, He C: **The *Xanthomonas oryzae* pv. *oryzae* PilZ domain proteins function differentially in cyclic di-GMP binding and regulation of virulence and motility**. *Appl Environ Microbiol* 2015, **81**:4358-4367.

35. Zhulin IB, Taylor BL, Dixon R: **PAS domain S-boxes in Archaea, bacteria and sensors for oxygen and redox**. *Trends Biochem Sci* 1997, **22**:331-333.

36. •• Guzzo CR, Dunger G, Salinas RK, Farah CS: **Structure of the PilZ–FimXEAL–c-di-GMP complex responsible for the regulation of bacterial type IV pilus biogenesis**. *J Mol Biol* 2013, **425**:2174-2197.
This paper presents the crystal structure of the *X. citri* 306 PilZ–$FimX_{EAL}$–c-diGMP complex and maps the effects of mutations of interfacing residues on stability and T4P-dependent phenotypes.

37. •• Yang F, Tian F, Li X, Fan S, Chen H, Wu M, Yang CH, He C: **The degenerate EAL-GGDEF domain protein Filp functions as a cyclic di-GMP receptor and specifically interacts with the PilZ-domain protein PXO_02715 to regulate virulence in *Xanthomonas oryzae* pv. *oryzae***. *Mol Plant Microbe Interact* 2014, **27**:578-589.
This study shows that the *X. oryzae* pv. *oryzae* FimX and PilZ homologs (Filp and PXO_02715, respectively) interact and their deletions result in reduced bacterial virulence and reduced T3SS gene expression.

38. •• Chin KH, Kuo WT, Yu YJ, Liao YT, Yang MT, Chou SH: **Structural polymorphism of c-di-GMP bound to an EAL domain and in complex with a type II PilZ-domain protein**. *Acta Crystallogr D: Biol Crystallogr* 2012, **68**:1380-1392.
This paper presents the crystal structure of the *X. campestris* pv. *campestris* 17 PilZ–$FimX_{EAL}$–c-diGMP complex and maps the effects of mutations of interfacing residues on protein complex stability.

39. Qi Y, Xu L, Dong X, Yau YH, Ho CL, Koh SL, Shochat SG, Chou SH, Tang K, Liang ZX: **Functional divergence of FimX in PilZ binding and type IV pilus regulation**. *J Bacteriol* 2012, **194**:5922-5931.

40. •• Ryan RP, McCarthy Y, Kiely PA, O'Connor R, Farah CS, Armitage JP, Dow JM: **Dynamic complex formation between HD-GYP, GGDEF and PilZ domain proteins regulates motility in *Xanthomonas campestris***. *Mol Microbiol* 2012, **86**:557-567.

In this study two-hybrid assays and *in vivo* FRET analysis was used to identify dynamic interactions between RpfG, two digyanylate cyclase proteins, a PilZ domain protein and the ATPases PilT and PilU.

41. Ryan RP, McCarthy Y, Andrade M, Farah CS, Armitage JP, Dow JM: **Cell-cell signal-dependent dynamic interactions between HD-GYP and GGDEF domain proteins mediate virulence in *Xanthomonas campestris***. *Proc Natl Acad Sci U S A* 2010, **107**:5989-5994.

42. Ryan RP: **Cyclic di-GMP signalling and the regulation of bacterial virulence**. *Microbiology* 2013, **159**:1286-1297.

43. Ryan RP, Fouhy Y, Lucey JF, Crossman LC, Spiro S, He YW, Zhang LH, Heeb S, Camara M, Williams P *et al.*: **Cell–cell signaling in *Xanthomonas campestris* involves an HD-GYP domain protein that functions in cyclic di-GMP turnover**. *Proc Natl Acad Sci U S A* 2006, **103**:6712-6717.

44. He YW, Xu M, Lin K, Ng YJ, Wen CM, Wang LH, Liu ZD, Zhang HB, Dong YH, Dow JM *et al.*: **Genome scale analysis of diffusible signal factor regulon in *Xanthomonas campestris* pv. *campestris*: identification of novel cell–cell communication-dependent genes and functions**. *Mol Microbiol* 2006, **59**:610-622.

45. • Guo Y, Zhang Y, Li JL, Wang N: **Diffusible signal factor-mediated quorum sensing plays a central role in coordinating gene expression of *Xanthomonas citri* subsp. *citri***. *Mol Plant Microbe Interact* 2012, **25**:165-179.
In this study, transcriptome analysis was used to characterize the DSF-mediated quorum sensing regulatory network in *Xanthomonas citri*.

46. • Barel V, Chalupowicz L, Barash I, Sharabani G, Reuven M, Dror O, Burdman S, Manulis-Sasson S: **Virulence and in planta movement of *Xanthomonas hortorum* pv. *pelargonii* are affected by the diffusible signal factor (DSF)-dependent quorum sensing system**. *Mol Plant Pathol* 2015, **16**:710-723.
This paper shows that the T4P-related genes *pilC* and *pilT* are strongly down-regulated in *Xanthomonas hortorum* pv. *pelargonii* mutant strains deficient in DSF-mediated quorum sensing.

47. Skotnicka D, Petters T, Heering J, Hoppert M, Kaever V: **Cyclic di-GMP regulates type IV pilus-dependent motility in *Myxococcus xanthus***. *J Bacteriol* 2015, **198**:77-90.

48. Andrade MO, Alegria MC, Guzzo CR, Docena C, Rosa MC, Ramos CH, Farah CS: **The HD-GYP domain of RpfG mediates a direct linkage between the Rpf quorum-sensing pathway and a subset of diguanylate cyclase proteins in the phytopathogen *Xanthomonas axonopodis* pv *citri***. *Mol Microbiol* 2006, **62**: 537-551.

49. •• Roelofs KG, Jones CJ, Helman SR, Shang X, Orr MW, Goodson JR, Galperin MY, Yildiz FH, Lee VT: **Systematic identification of cyclic-di-GMP binding proteins in *Vibrio cholerae* reveals a novel class of cyclic-di-GMP-binding ATPases associated with type II secretion systems**. *PLoS Pathog* 2015, **11**:e1005232.
This paper shows that c-di-GMP binds directly to ATPases associated with Type II secretion systems and Type IV pili in some bacterial genera.

50. Bordeleau E, Purcell EB, Lafontaine DA, Fortier LC, Tamayo R, Burrus V: **Cyclic di-GMP riboswitch-regulated type IV pili contribute to aggregation of *Clostridium difficile***. *J Bacteriol* 2015, **197**:819-832.

51. Henrichsen J: **Twitching motility**. *Annu Rev Microbiol* 1983, **37**:81-93.

52. Lim SH, So BH, Wang JC, Song ES, Park YJ, Lee BM, Kang HW: **Functional analysis of pilQ gene in *Xanthomonas oryzae* pv. *oryzae*, bacterial blight pathogen of rice**. *J Microbiol* 2008, **46**:214-220.

53. Harshey RM: **Bacterial motility on a surface: many ways to a common goal**. *Annu Rev Microbiol* 2003, **57**:249-273.

54. Ryan RP, Fouhy Y, Lucey JF, Jiang BL, He YQ, Feng JX, Tang JL, Dow JM: **Cyclic di-GMP signalling in the virulence and environmental adaptation of *Xanthomonas campestris***. *Mol Microbiol* 2007, **63**:429-442.

55. McCarthy Y, Ryan RP, O'Donovan K, He YQ, Jiang BL, Feng JX, Tang JL, Dow JM: **The role of PilZ domain proteins in the virulence of *Xanthomonas campestris* pv. *campestris***. *Mol Plant Pathol* 2008, **9**:819-824.

56. Dow JM, Crossman L, Findlay K, He YQ, Feng JX, Tang JL: **Biofilm dispersal in *Xanthomonas campestris* is controlled by cell–cell signaling and is required for full virulence to plants**. *Proc Natl Acad Sci U S A* 2003, **100**:10995-11000.

57. Jacques MA, Josi K, Darrasse A, Samson R: ***Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* is aggregated in stable biofilm population sizes in the phyllosphere of field-grown beans**. *Appl Environ Microbiol* 2005, **71**:2008-2015.

58. Malamud F, Torres PS, Roeschlin R, Rigano LA, Enrique R, Bonomi HR, Castagnaro AP, Marano MR, Vojnov AA: **The *Xanthomonas axonopodis* pv. *citri* flagellum is required for mature biofilm and canker development**. *Microbiology* 2011, **157**:819-829.

59. Li J, Wang N: **Genome-wide mutagenesis of *Xanthomonas axonopodis* pv. *citri* reveals novel genetic determinants and regulation mechanisms of biofilm formation**. *PLoS One* 2011, **6**:e21804.

60. Kraiselburd I, Alet AI, Tondo ML, Petrocelli S, Daurelio LD, Monzon J, Ruiz OA, Losi A, Orellano EG: **A LOV protein modulates the physiological attributes of *Xanthomonas axonopodis* pv. *citri* relevant for host plant colonization**. *PLoS One* 2012, **7**:e38226.

61. Chae JC, Hung NB, Yu SM, Lee HK, Lee YH: **Diversity of bacteriophages infecting *Xanthomonas oryzae* pv. *oryzae* in paddy fields and its potential to control bacterial leaf blight of rice**. *J Microbiol Biotechnol* 2014, **24**:740-747.

62. Ahern SJ, Das M, Bhowmick TS, Young R, Gonzalez CF: **Characterization of novel virulent broad-host-range phages of *Xylella fastidiosa* and *Xanthomonas***. *J Bacteriol* 2014, **196**: 459-471.

63. Balogh B, Dickstein ER, Jones JB, Canteros BI: **Narrow host range phages associated with citrus canker lesions in Florida and Argentina**. *Eur J Plant Pathol* 2013, **135**:253-264.

64. Ahmad AA, Ogawa M, Kawasaki T, Fujie M, Yamada T: **Characterization of bacteriophages Cp1 and Cp2, the strain-typing agents for *Xanthomonas axonopodis* pv. *citri***. *Appl Environ Microbiol* 2014, **80**:77-85.

65. Ahmad AA, Askora A, Kawasaki T, Fujie M, Yamada T: **The filamentous phage XacF1 causes loss of virulence in *Xanthomonas axonopodis* pv. *citri*, the causative agent of citrus canker disease**. *Front Microbiol* 2014, **5**:321.

66. Lee CN, Tseng TT, Chang HC, Lin JW, Weng SF: **Genomic sequence of temperate phage Smp131 of *Stenotrophomonas maltophilia* that has similar prophages in xanthomonads**. *BMC Microbiol* 2014, **14**:17.

67. Wang L, Makino S, Subedee A, Bogdanove AJ: **Novel candidate virulence factors in rice pathogen *Xanthomonas oryzae* pv. *oryzicola* as revealed by mutational analysis**. *Appl Environ Microbiol* 2007, **73**:8023-8027.

68. Das A, Rangaraj N, Sonti RV: **Multiple adhesin-like functions of *Xanthomonas oryzae* pv. *oryzae* are involved in promoting leaf attachment, entry, and virulence on rice**. *Mol Plant Microbe Interact* 2009, **22**:73-85.

69. Persat A, Inclan YF, Engel JN, Stone HA, Gitai Z: **Type IV pili mechanochemically regulate virulence factors in *Pseudomonas aeruginosa***. *Proc Natl Acad Sci U S A* 2015, **112**:7563-7568.

70. Facincani AP, Moreira LM, Soares MR, Ferreira CB, Ferreira RM, Ferro MI, Ferro JA, Gozzo FC, de Oliveira JC: **Comparative proteomic analysis reveals that T3SS, Tfp, and xanthan gum are key factors in initial stages of *Citrus sinensis* infection by *Xanthomonas citri* subsp. *citri***. *Funct Integr Genomics* 2014, **14**:205-217.

71. Darsonval A, Darrasse A, Durand K, Bureau C, Cesbron S, Jacques MA: **Adhesion and fitness in the bean phyllosphere and transmission to seed of *Xanthomonas fuscans* subsp. *fuscans***. *Mol Plant Microbe Interact* 2009, **22**:747-757.

72. Qian W, Jia Y, Ren SX, He YQ, Feng JX, Lu LF, Sun Q, Ying G, Tang DJ, Tang H *et al.*: **Comparative and functional genomic analyses of the pathogenicity of phytopathogen *Xanthomonas campestris* pv. *campestris***. *Genome Res* 2005, **15**:757-767.